STUDIES ON PLANT SIGNALS AND GROWTH RESPONSES TO HERBIVORY AND ITS SIMULATION

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Certificate

This is to certify that the thesis entitled, ***"Studies on plant signals and growth responses to herbivory and its simulation"****, submitted for the degree of* ***Doctor of Philosophy*** *in* ***Botany*** *is a faithful record of the bonafide research work carried out at Aligarh Muslim University, Aligarh, India, by* ***Ms. Farha Rehman*** *under my guidance and supervision and that no part of it has been submitted for any other degree or diploma.*

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Research Supervisor

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Chapter 1

Introduction

INTRODUCTION

Plants during their life time are exposed to a variety of abiotic and biotic stresses. The biotic stress encompasses diseases and phytophagous pests including insect herbivory. Insect pests causes major biotic stress to agricultural crops. Plants have evolved varying defense mechanisms to resist these biotic stresses. Insect herbivory leads to the activation of various defense mechanisms in host plants resulting in qualitative and/or quantitative changes in plant production owing to alteration in metabolic processes. The diverse distribution of insect herbivores among their host plants and its effect on the growth and defense strategies of plants has long been a topic of interest for ecologists. Insects have been the most significant herbivores and the evolution of defense mechanism in land plants switched the co-evolution of counter defenses in insects. Herbivory also induces a unique plant defensive strategy by promoting the activity of natural enemies of the herbivores. Insects are the primary herbivores in many ecosystems and feed upon a vast variety of plants ranging from algae to angiosperms. From an agricultural point of view herbivorous insects are considered major pests and held responsible for substantial crop losses (Schoonhoven et al., 1998; 2005; Ferry et al., 2006). About 80 % of the plant materials consumed by insects and their secondary production can equal or exceed that of more conspicuous vertebrate grazers in grassland (Anonymous, 2008; Farha-Rehman et al., 2012b).

Indian mustard (*Brassica juncea* L. Czern. & Coss) belongs to the family Brassicaceae (formerly Cruciferae) of flowering plants. The plant is erect, green annual herb of one to two meter height. Foliages are pale green with few hairs (pubescent) on first few leaves. Leaf blades extend up to petioles. The lower leaves are deeply notched while upper leaves are narrow and entire; flowers are small yellow with petals arranged diagonally. In Indian sub-continent *Brassica juncea* is the dominant oilseed crop (Prakash, 1980). Mustard cultivation has gained wider acceptance among farmers due to its adaptability to both irrigated and rainfed areas.

Oil seed crops in India constitutes about 13 % of the area under agricultural practices and contribute about 5 % to the gross national product and 1 % value of all agricultural products. India has the distinction of being the world's largest oilseed

growing country sharing 25.6 million hectares out of the total 125 million hectares of land under oilseed cultivation in the entire world. Mustard accounts for 10 % of the total world production (Downey and Rimmer, 1993). Insect pests (including aphids) are among one of the major factors of yield losses. The most voraciously attacking and reproducing aphids of mustard crop includes *Lipaphis erysimi* (Kaltenbach), *Brevicoryne brassicae* (L.) and *Myzus persicae* (Sulz.). These three aphid species account for about 70-80 % of oil crop losses (Mandal et al., 1994; Swati, 2005). Among these, mustard aphid (*L. erysimi,* Kalt.) is the key pest of Brassicaceae family. Severe infestation of this pest often leads to 35-96% loss or complete loss of the mustard crop (Bakhetia, 1986; Bakhetia and Sekhon, 1989; Choudhury and Pal, 2009). In Aligarh, Indian mustard aphid (*L. erysimi*) was found most common and notorious attacker on different cultivars of mustard crops grown (Farha-Rehman et al., 2013).

Herbivory is an act of consumption of plant biomass and nutrients by animals or insects. This key ecosystem process regulates the flow of energy from producers to consumers (Farha-Rehman et al., 2010). Herbivory affects primary production, vegetation structure and composition in terrestrial and aquatic ecosystems and influences a variety of ecosystem properties primarily through differential changes in survival, productivity, growth and composition of the host plants (Anonymous 2008; Farha-Rehman et al., 2012b). The vascular plants evolved various defense strategies against herbivores but a number of sap-sucking, leaf mining, gall forming herbivores and nectar feeding insects co-evolved counter defenses (Anonymous, 2008; Farha-Rehman et al., 2012b). The study of plant defenses against herbivory is not only important from evolutionary view point but it is also useful in understanding the extent of its impact on agriculture, human and livestock food sources as well as utility and survival of plants.

The plant defenses against herbivory are either constitutive or induced. Constitutive defense are ready-chemical defensive arsenals like routine sequestering and accumulation of digestibility reducers and toxins, etc. The host plants may also respond to herbivory by changes in morphological characteristics such as trichomes, spines, thorns and hairs. *Brassica juncea* possesses trichomes as a constitutive morphological defense strategy. These are known to affect the feeding behaviour and

performance of some herbivores at various stages of their lives (Fernandes, 1994; Traw and Dawson, 2002; Agrawal and Fishbein, 2006; Mathur, 2012). The induced defense includes sequestering of secondary metabolites and morphological and/or physiological changes in host plants after herbivore infestation. Herbivory and mechanical wounding triggers the major secondary metabolites to re-sequester and produce defensive metabolites with low molecular weight (Karban and Baldwin, 1997). These two ways of plant defense (inducible and constitutive), together increase defensive ability and effectiveness of host plants against herbivory (Anonymous, 2008; Farha-Rehman et al., 2012b). The plants also possess 'direct defense barriers' which include thick cuticle, trichomes and thorns. The chemical defenses include sequestering of toxins, repellents and digestibility reducers (van Poecke and Dicke, 2002). Defensive chemicals can be found in all major classes of plant secondary metabolites which are secreted after herbivore attack. These chemicals include nitrogen-containing metabolites like alkaloids and glucosinolates, phenolics like phenylpropanoids and flavonoids as well as terpenoids in addition to defense-related proteins (van Poecke and Dicke, 2002).

Glucosinolates (GS) are the main secondary metabolites in brassicaceous plants. The hydrolyzed products of glucosinolates mediated by enzyme myrosinase play important roles in plant defense and plant-insect communication in several members of crucifers (Hopkins et al., 2009; Khan et al., 2010). Formation of toxic isothiocynates (ITC) is the outcome of defense system in the glucosinolate-myrosinase cycle of family Brassicaceae. It is activated upon tissue damage caused by the herbivore. Isothiocyanates are degradation products of glucosinolates in mustard plants and have repellent effect on phytophagous insects. But lepidopteran herbivores specialized on Brassicaceous plants possess a co-evolved biochemical adaptations to inhibit the process of isothiocyanates formation. Allyl isothiocyanate (AITC) not only repel attacking insects and other herbivores through its volatilization but also induce stomatal closure in *Arabidopsis* with the production of reactive oxygen species (ROS) and nitric oxide (NO) and elevation of cytosolic Ca^{2+} (Khokon et al., 2011). These results raise the possibility that crucifer plants produce ITCs in response to herbivory and induce stomatal closure leading to suppression of water loss and invasion of fungi through stomata (Khokon et al., 2011). The secondary metabolites glucosinolate-

myrosinase system, therefore, plays a key role in plants defense mechanism, at least in mustard plants. Some plant volatiles like phytoalexins, phytoanticipins, sulphur lectins and numerous other classes of secondary metabolites such as hydroxamic acids, alkaloids, terpenes and C-6 aldehydes are known for their insecticidal activities (Ahuja et al., 2010; Atri et al., 2012).

The indirect plant defenses involve the attraction of predators through chemical signaling and thereby triggering their attraction to check and balance the population of herbivores (De Vos and Jander, 2010). The specialist predators or parasites of herbivores are signaled through volatile chemicals. Synthesis and release of these chemical signals are triggered by chemical elicitors or substances contained in the oral secretion of herbivores. Certain chemicals contained in the saliva of grazing insects (herbivores) activate the synthesis and release of the plant specific blends of volatiles with high sensitivity to receptor molecules of predators (De Moraes et al., 2001). Some of these volatile compounds provide important host location cues to predator insects or parasites that are natural enemies of herbivores (De Moraes et al., 2001; Farha-Rehman et al., 2010).

Predatory insects are often attracted on simulation of herbivory like application of jasmonic acid (JA) and mechanical injury by leaf clipping or their combined effects. Such herbivory simulations often respond like signaling of natural herbivory (van Kleunen et al., 2004). For instance, the volatile compound, AITC have multiple roles viz. (a) it repels attacking aphids (b) signal the predatory beetles (*Coccinella septempunctata*) and (c) induces stomatal closure as part of indirect defense (Khokon et al., 2011). Many other plants also initiate indirect defenses through the release of volatiles to attract ladybugs; parasitoid wasps while some other aphids consuming predatory ladybirds/beetle like *Coccinella septumpunctata* (De Vos and Jander, 2010). Ladybird (*Coccinella septumpuctata)* is a well known beetle predating upon a wide range of aphids including *L. erysimi* and found in many habitats like fields, gardens, forest sea coast, mountains and cities (Hodek and Honek, 1996; Ali and Rizvi, 2009). Both, adults and larvae of ladybird feed on a variety of other soft bodied herbivorous pests; viz. whiteflies, adelgids psyllids, mealy bugs (Ali and Rana, 2010).

The host plants vary with genetic variability of cultivars, environment and physiological status (Atri et al., 2012). To a given stress plants show specific chemical response from out of complex integrations of many blends of chemicals produced in the plant. These chemical defenses of plant against herbivory are always at the cost of photosynthates and in turn, the plant growth and yield. In India and North Europe aphids are known for incurring large scale damage to plants either directly by feeding or by transmitting viral diseases through their stylet (Sekhon, 1999). In India aphids are known to feed on mustard crop from vegetative to fruiting stages and cause severe qualitative and quantitative crop losses (Sekhon, 1999). The crop is damaged maximum at flowering stage due to its high susceptibility at this stage and the prevailing weather regimes being more conductive to aphid multiplication (Bakhetia and Brar, 1983).

Aphids with a diversity of 4000 species (worldwide) form a largest group of phloem feeding insects (Bak et al., 2013). The aphids, by direct phloem sap sucking remove nutrients from out of plants reserve carbon and cause growth stunting of host plants and alters source-sink carbon allocation patterns (Blackman and Eastop, 1994). Aphid infestation sometimes also causes gall-formation, chlorosis, necrosis, wilting in addition to other growth malformations (Guerrieri and Digilio, 2008; Morkunas et al., 2011).

The responses of host plant to aphid attack have been focused in the present work. Aphids ingest phloem sap from plants through narrow piercing–sucking stylets. This mechanism of aphid starts with the probing of suitable host organ, piercing stylets in epidermal mesophyll and parenchyma cells. This mechanical damage by insect stylets may trigger plant's chemical defense response to infestation (Tjallingii and Esch, 1993; Goggin, 2007). Long term aphid probing or insect saliva may induce changes in the chemical nature of the sieve element sap (Telang et al., 1999; Ponder et al., 2001). Aphids are major agricultural pests because of their unparalleled reproductive capacity and ability to manipulate host plant's defensive physiology (Chugh et al., 2013). Aphids feed and suck water and nutrients from the phloem. After damage of the plant tissues, the toxins of the aphid saliva cause thickening, crumpling, and downward curling of leaves (Mossler, 2005; Ahuja et al., 2010). Adult aphids and nymphs are mobile and frequently change their feeding sites several times

during their lifetime. The direct consumption of phloem sap by nymphs and adults from leaves, stem and flowers reduce the pod formation and oil content in grains up to 75 per cent (Sekhon, 1999). The aphid saliva is also toxic to plant tissues. Morkunas et al. (2008) reported that aphids enter their stylet in plant tissues primarily via intracellular route and inject saliva. Miles and Peng (1989) found that aphid saliva was extremely toxic to plant tissues around the stylet tracks. The aphid saliva disrupted chloroplast and induced hormonal disbalance in the plants (Morkunas et al., 2008). Aphids secrete a proteinaceous salivary sheath lining the stylet path. The watery saliva of aphid contains numerous enzymes such as oxidases, pectinases and cellulases which cause cell wall breakdown and help aphids to penetrate their stylets easily in plant tissues (Goggin, 2007).

Prolonged aphid infestations can cause premature leaf abscission and induce defoliation of host plant (Rosenheim et al., 1997). About half of all insect species are herbivores and are also responsible for about 5-10 % and often 10-30 % leaf defoliation every year (Schoonhoven et al., 1998). The loss of leaf area reduced shoot biomass, number of stem nodes and photosynthesis (Bagwell et al., 1991; Layton et al., 1996; Rosenheim et al., 1997).

The plant growth regulators such as JA also play an important role in plant defence against insect attack. Jasmonic acid induces some volatiles to attract predator insects to feed upon herbivorous insect. Besides these, JA also secures plant growth and development (Rohwer and Erwin, 2006). The JA mediated signaling to predatory insect is activated by phloem feeding aphids. But very little is known about the specific impact of JA on the expression of genes that respond to aphid attack (Kuśnierczyk et al., 2011).

Jasmonic acids are synthesized through the activation of octadecanoid pathway and regulate various physiological processes in plants such as pollen maturation, tendril coiling, and senescence in addition to plant defense (Creelman and Mullet, 1997; Steppuhn and Baldwin, 2008). The JA and its volatile methyl ester (MeJA) act as endogenous regulator of wound-induced chemistry and signal molecules on insects attack (Baldwin, 1999; Karban et al., 1999; Thaler, 1999a). Jasmonic acids and its metabolites serve as phloem-mobile long-distance signals and

activate the expression of defense genes in distal plant parts, physical and chemical defense traits through defense signaling (Howe and Schaller, 2008).

External application of JA as spray can induce the emission of a blend of volatiles from plants often similar to responses induced during herbivore feeding. Interestingly, Heil (2004) found minimal induction of volatile emission in *Phaseolus lunatus* by JA led to secretion of extra floral nectar (EFN) and stronger induction of herbivore repelling tendency. Such an understanding of the role of JA in tri-trophic interactions may help in effective use of natural predatory beetles as a facilitator of biological control of herbivory. Ladybug *Coccinella septempunctata* is a predatory beetle (at 3rd trophic level) which feeds on herbivorous aphids. It is attracted with the emission of allyl isothiocyanate or AITC. It is further reported that natural herbivory on *Macaranga tanarius* (L.) Müll. Arg. was reduced by the application of JA (Heil et al., 2001).

Each plant species has its own unique set of chemical defense and induction of defenses should not always be attributed to jasmonate application alone. Such reponses of JA should also not be used to conclude that the plants will be better defended against pests. JA-induced defenses such as protease inhibitors (PI) may often lead to the production of PI-resistant digestive enzymes in herbivores without affecting herbivore performance (Broadway, 1995; 2000).

Extra floral nectar induced by JA attracts beneficial insects to plants besides reduction in herbivore numbers and damage (Arimura et al., 2005; Heil et al., 2001; Linsenmair et al., 2001). Similar results were observed in *Phaseolus lunatus* (Heil, 2004). The use of pesticides in agriculture and horticulture directly threatened natural ecosystem. The targeted use of jasmonate-induced defenses may provide valuable augmentation of integrated pest management strategies in agriculture and horticulture. For example, jasmonates may be used to treat localized infestations where a pest threshold is exceeded with the goal of attracting predators or parasitoids.

It is very important to explore the mechanisms of plant defense against aphids and to identify the factors that regulate resistance or susceptibility of the host plants. In the present study aphid and JA induced responses of mustard-aphid-beetle (tri-trophic model) have been studied. The direct and indirect plant defense system (biochemical, physical, histological and physiological) etc. have been studied in

responses to aphid infestation and its simulation through JA application. It is believed that plant response to aphids is complicated involving several defense strategies (Thompson and Goggin, 2006). This work examines the effect of aphid infestation (natural herbivory) and application of JA (simulated herbivory) on physiological and biochemical alterations in the host plant resultant adaptation to resist aphid infestation and the behavior of predatory beetle.

The present work was carried out with the following objectives:

1. Screening of five cultivars of mustard (*Brassica juncea*) to work out least sensitive and most susceptible cultivars response to a constant number of aphid (*Lipaphis erysimi*).
2. To compare the response of screened least sensitive and most susceptible cultivars of mustard to varying levels of aphid infestation.
3. To find out the effects of predatory beetle (*Coccinella septempunctata*) on different levels of aphid population infested on two selected mustard cultivars
4. To compare the relative responses of two screened cultivars of mustard to varying concentration of JA applications (as simulation of aphid infestation).
5. Combined effect of simulated herbivory (JA) and natural herbivory (aphid) on resistant and susceptible cultivars of mustard.

Chapter 2

Review of Literature

Plants face many abiotic and biotic challenges during their life time. The abiotic stresses include drought, flooding, temperature fluctuations and metal stress. However, attack by different kinds of organism, viz. parasites and herbivores are major biotic challenges for the plant. Herbivory is the act of consumption of specific plant parts like foliage, stem, root, flower, fruit or seeds by animals or insects (Bruinsma and Dicke, 2008). Herbivory depends on the type and intensity of feeding habit of herbivore. It is a key ecosystem process through which energy is transferred from autotrophs to heterotrophs (Anonymous, 2008; Farha-Rehman et al., 2012b). This process also reduces density of plants, and transfers part of biomass and nutrients to the soil (Anonymous 2008; Farha-Reman et al., 2012b). Insects like aphids and whiteflies feed on the plant sap while spider-mites and thrips feed on the epidermal or mesophyll cell contents of the leaves (Walling, 2000). About 950,000 insect species contribute almost 56% of the diversity of animal kingdom. Of these, about 9000 species of insects are pests and incur major crop losses (Grisworld, 1953). Severe crop damages by a number of insect herbivores are reported from developed and developing nations. There are conflicting reports on crop losses by insects. The global crop damage by insects vary between 35-37% according to one estimate (Atwal and Dhaliwal, 2003; Farha-Rehman et al., 2010) and 10-20% by another estimate (Ferry et al., 2003). Insects caused 10-30% annual crop losses in North America, Europe and Japan, (Atwal and Dhaliwal, 2003). In accordance with their density grass hoppers (*Choreodoeus illustris*) could consume 32-79% leaves of *Zea mays* plants (Farha Rehman, 2008).

Aphids belong to the family Aphidoidea within order Hemiptera. Aphids are nefarious plant pests, especially on the members of family Brassicaceae and cause damage to crop plants by sucking plant sap and transmitting pathogenic viruses (Bhatia et al., 2011). A slender stylet bundle constitutes the modified mouth parts of the aphids. The stylet punctures the leaf surface and then penetrates predominantly through middle lamella to reach the sieve element and suck-in the nutrient-rich phloem sap (Kaloshian and Walling, 2005). Large concentration of carbohydrates in the phloem sap create an osmotic imbalance in the aphid gut (Walling, 2008). To

avoid this dehydration, aphids maintain their water balance by occasional feeding through the xylem (Spiller et al., 1990).

Various species of Brassica are important edible oil crops of India. *Brassica juncea* (Indian mustard, locally called 'rai') is the major oil yielding crop among other species of Brassica of family Brassicaceae (Bhatia et al., 2011). The plants, during growth are exposed to various biotic (herbivory, fungal, bacterial) stresses and enhanced the synthesis of primary and secondary metabolites. In this process, a number of defensive signaling viz. salicylic acid (SA), jasmonic acid (JA), ethylene and abscisic acid pathways are activated in the plant (Zhao et al., 2007). The systemically induced defense responses of *Brassica* species might use complex defensive mechanisms than a common set of biosynthetic pathways (Jahangir et al., 2009). Plants under diverse natural stresses are forced to evolve more co-ordained rather than conflicting defense strategies (Bruce and Pickett, 2007). In case of aphid infestation, composition of saliva and attacking mechanism, activates selected gene expression and blocks specific sites of a metabolic pathway, or even metabolize the plant defense compounds (Jahangir et al., 2009).

Among the biotic stresses, damage caused by aphids is considered as a major constraint in the growth and productivity of these crops (Bhatia et al., 2011). Different species of *Brassica* are infested by a variety of aphid species such as green peach aphid (*Myzus persicae*), cabbage aphid (*Brevicoryne brassicae* L.) and mustard aphid (*Lipaphis erysimi* Kalt.), as reported by Bhatia et al. (2011). Indian mustard (*Brassica juncea)* is predominantly infested by *L. erysimi* (Atri et al., 2012). All the growth stages of the crop were attacked by aphids but the greatest damage was done during the flowering and pod formation stages (Bakhetia, 1991; Bhatia et al., 2011). Retarded growth, poor seed formation and low oil content are the prominent manifestations of aphid feeding (Malik and Anand, 1984; Bakhetia, 1987; Atri et al., 2012; Louis and Shah, 2013). Both the nymphs and adult aphids devitalize crop by sucking the cell sap (Bakhetia, 1991; Atri et al., 2012). Mustard aphid (*L. erysimi* Kalt.) is one of the most damaging pests that confronts this crop and is highly host specific, feeding exclusively on *Brassica* phloem sap (Bhatia et al., 2011).

Plant defense strategies against herbivory

The plants evolved a broad array of constitutive and induced defenses against herbivores. Some of these are morphological, and some others chemical in nature (Vickers, 2011). Constitutive plant defenses include glandular trichomes, cuticular waxes, (Wittstock and Gershenzon, 2002) and other structural and chemical defenses like cell wall modification, synthesis of proteins, secondary metabolites of toxic nature and predator inviting volatiles (Goggin, 2007). Inducible chemical defenses include sequestering of a wide variety of toxic, anti-nutritive, or injurious compounds to repel attacking organisms. These defensive compounds include alkaloids, phenolic compounds, chitinases, and protease inhibitors (Rohwer and Erwin, 2008).

The defense strategies of some plants to herbivory include avoidance and tolerance by some plants through diversion of resource allocation to damaged parts (Vickers, 2011). In some other plants, defense strategy includes induction of R gene or activation of signalling pathways (Howe and Jander, 2008; Vickers, 2011). The antixenosis (deterring effect) and antibiosis (toxic for survival) are chemical defenses (mainly secondary metabolites) of plants (Parsa et al., 2011; Vickers, 2011). The induced defenses increased the plant fitness in natural environment as in *Raphanus raphanistrum* (Agrawal, 1999).

The air born volatile signals constitute defensive chemicals (aldehyde, alcohols and esters) that protect plants from insects and induce intact undamaged neighbouring plants to produce and emit sesquiterpenes and JA (Engelberth et al., 2004). The immunity of plant to insect herbivory is caused by recognising insect attack and through released volatile signals from injured cell of neighbour plant (Howe and Jander, 2008).

Constitutive plant defenses

Plants have evolved many constitutive defense traits to deter herbivores. Some of the major constitutive morphological plant defense is appended as follows.

Trichomes

Trichomes are the hair like epidermal appendages and are produced by most plant species (Werker, 2000). The leaf trichomes serve defensive functions like protection and resistance against herbivores (Levin, 1973; Dalin et al., 2008). In many plant species, trichome density in new leaves increased after herbivore infestation

(Levin, 1973). But, this constitutive adaptation is also affected by the abundance and effectiveness of predators and parasitoids feeding on herbivore (Levin, 1973). Trichomes are composed of cellulose and other substances that constitute low nutritional value for the insects.

The trichomes vary in shape, size and cellular organisation (Southwood, 1986; Werker, 2000). Some glandular trichomes release secondary metabolites (e.g. terpenes and alkaloids) which can be poisonous, repellant, or may trap insects or other organisms (Duffey, 1986; Hare and Elle, 2002; Rautio et al., 2002). The herbivores induced production of new leaves with higher density of trichomes and consequently the foliage consumption by insect fell down (Agrawal, 1999, 2000; Dalin and Bjorkman, 2003). Trichomes influence insect oviposition and/or feeding in a wide range of insects and other herbivores (Levin, 1973). Non-glandular trichomes mainly function as a structural defense against small herbivores (Levin, 1973; Karkkainen et al., 2004). The trichomes interfered with the movement of insects on the leaf surface making accessibility to the leaf epidermis difficult for feeding (Southwood, 1986). The trichomes are relatively soft 'weapons' in plant defense against herbivory compared to other lethal trait (Dalin et al., 2008). But their presence on host plant influences both selection behavior and population growth of herbivorous insects (Dalin et al., 2008). These studies suggest that the trichomes protect host plant from herbivorous insects.

Leaf trichomes also influenced the performance of herbivore predators. This may indirectly affect the strength of damage caused by herbivores (Dalin et al., 2008). The trichomes may have a neutral, negative or positive effect on predators (Dalin et al., 2008). Both non-glandular and glandular trichomes may have any of these effects on predators (Obrychi and Tauber 1984; Romeis et al., 1994; Styrsky et al., 2006). The tiny hooked trichomes on leaves and stems of *Mentzelia punila* (Family Loasaceae) have a detrimental effect on plants against herbivores (Eisner et al., 1998; Farha-Rehman et al., 2010). The hooked trichomes adversely affected both herbivore aphids (*Macrosiphum mentzeliae*) and its predator, Coccinellid beetle; *Hippodamia convergens* (Eisner et al., 1998; Farha-Rehman et al., 2010).

Cuticle

Cuticle being first contact zone poses a potent resistance to insects (Samuels et al., 2008; Muller, 2008). Chemical compounds in the cuticle deter the herbivores directly or have a toxic effect (Muller, 2008; Yeats et al., 2013). Herbivores induced an additional wax production and biosynthesized secondary metabolites that are deposited at the plant cuticle (Muller, 2008).

Aphid attack on *Beta vulgaris* L. (Chenopodiaceae) induced wax production (Bystrom et al., 1968). But, crystalline epicuticular wax reduced in the leaves of *Sorghum halepense* infested by *Sipha flava* (Muller, 2008). The infested leaves of plant became even more susceptible to the aphids than intact plant (Gonzales et al., 2002). Cuticular lipids perform important function in multitrophic interactions, as their major constituents can be chemically very similar between cuticles of plants, herbivores, and their predators (Muller, 2008).

Wound periderm

Franceshi et al. (2005) and Ginzberg (2008) observed the role of periderm in plant defense mechanism. The formation of wounded periderm at the boundaries of the damaged region to isolate it from non-wounded healthy tissue is one of the defensive strategies of the plant. The wound periderm may prevent from successive pest invasions and fluid loss (Ginzberg, 2008; Ichihara et al., 2000).

The purpose of wound healing in plants after herbivory is to minimize the pathogen invasion and fluid loss (Ginzberg, 2008). Periderm is a secondary protective tissue and replaces the damaged epidermis. The inner cell layers of potato tuber periderm produce high levels of glycoalkaloids, which are toxic secondary metabolites that are active against pests and pathogens (Krits et al., 2007). The suberin is the main protective substance deposited in outer wall layers of its cell wall (Ginzberg, 2008). The rate of establishment of suberized periderm following injury (e.g. after aphid feeding) is an important factor in the plant resistance to indirect damage following the wounding such as water loss (Ginzberg, 2008). He also noticed that periderm formation and their suberization are considered as generalized responses to wounding. However, developmental stages, biosynthetic pathways of periderm formation and its suberization are not yet completely known (Ginzberg, 2008).

Chemical Defenses

Antibiosis and antixenosis are widespread defense mechanisms of plants against aphid herbivory. Some of these are constitutive and others induced one as reported by Parsa et al. (2011) and Louis and Shah (2013). Inducible antibiosis defense has been demonstrated recently in *Arabidopsis thaliana* in response to feeding by the aphid *Myzus persicae* (Louis and Shah, 2013). Plants convert indole glucosinolate and a secondary metabolite (indol-3 allylmethylglucosinolate) into a more toxic 4-methoxyindol-3-ylmethylglucosinolate to defend the injured plants. This induced defense was localized and not systemic (Kim and Jander, 2007). The sequestered toxic compounds in a resistant genotype of soybean, subsidized the feeding and expanded maturation periods of *Aphis glycines matsumur* (Li et al., 2004). The secondary defensive metabolites including saponins, act as feeding deterrent to pea aphid *Acyrthosiphon pisum* and *A. pisum*, which reduce aphid's ability to ingest phloem or xylem sap (Golawska, 2007). Saponins also reduce the growth and reproductive rates of *A. pisum* (Sylwia et al., 2006). Besides these wide spread defensive chemicals in leaf tissues affecting feeding abilities of aphids, there are specific localized deterrents in the phloem (Vickers, 2011). Aphids are phloem feeders, and cause mechanical tissue damage while inserting their stylets and hence pose detection problems for the host plants (Louis and Shah, 2013). Phloem based defense is a specialized trait induced only in phloem feeding aphids. This strategy save resource allocation costs and initiates only minimum plant defense upon aphid infestation (Walling, 2008).

A study on the resistant breeding line of melon (*Cucumis melo*, AR5) affirmed that resistance against the cotton-melon aphid was located within the sieve elements and controlled aphid population by reduced phloem ingestions as well as longer salivation period (Klingler et al., 1998). These results were possible due to induced phloem defense traits in resistant lines of melon. In a later study Will and van Bel (2005) revealed that aphids must have thin and strong stylet long enough to reach and puncture the sieve tubes at a particular site. Against the disturbance of these kinds, the sieve tubes in angiosperms possess elaborate sealing mechanisms such as protein plugging and callose sealing which are triggered by a rise in calcium in the sieve tubes (Giordanengo et al., 2010). The Ca^{2+} influx in sieve element is mechano-sensitive and seems to be important for phloem occlusion in response to aphid

infestation as well (Knoblauch et al., 2001; Furch et al., 2009; Khokon, 2011). A limited cell death around the site of stylet impregnation has also been recorded by Pegadaraju et al. (2007) and Girling et al. (2008). Further, it is investigated that the plant biochemical wound response pathways are involved in the production of aphid-induced plant volatiles. The infestation of *Arabidopsis thaliana* by peach–potato aphid (*Myzus persicae)*, volatile production via octadecanoid pathways and activation of COI1 gene was induced (Girling et al., 2008; Louis and Shah, 2013). In the case of arthropod feeding, changes in plant metabolism and gene expression were associated with both, the general plant defense responses and specific aphid resistance gene (Moran and Thompson, 2001).

Plant-aphid interaction may activate dual defensive pathways; one species specific and another general response; common for many plant aphid interactions. The feeding of *Diuraphis noxia* (Mordvilko) and *Myzus nicotianae* on wheat and tobacco induced increased expression of glutamate synthatase, an enzyme produced and deployed in response to many cellular stresses (Walling, 2000; Moran and Thompson, 2001; Smith and Boyko, 2007) .

The predator recruitment through chemical signaling is unique defensive strategy of plant (Farha-Rehaman et al., 2012a,b; Vickers, 2011). Plants attracted predatory insects through a special blend of volatile chemical signalling. Several types of secondary metabolites play an important role against insect attack, for instance; glucosinolates accumulate in Brassicaceae family on aphid herbivory (Kazana et al., 2007; Ahuja et al., 2010). The glucosinolates are toxic to aphid herbivores and its degraded isothiocyanate compounds (in the presence of myrosinase enzyme) signal the natural enemies of herbivores (Kazana et al., 2007; Ahuja et al., 2010). Infested turnip plants release higher levels of isothiocyanates than uninfested plants, promoting attraction of *Diaeretiella rapae* a predator (Blande, 2004).

Role of jasmonic acid against aphid feeding

Jasmonic acid is biosynthesized (through octadecanoid pathway) from polyunsaturated fatty acids via a series of enzymatic reactions and released from chloroplast membranes (Meyer et al., 1984). Allene oxide synthase (AOS) gene encodes an enzyme to synthesise 12-oxophytodienoic acid (OPDA), a precursor for the synthesis of JA (Park et al., 2002). Several genes whose products are involved in

JA biosynthesis or JA-dependent signalling are up-regulated and thus, JA-derived compounds regulate gene expressional changes (Kehr, 2006). As a result of transcriptional reprogramming, the production of proteins involved in defense is promoted (Kehr, 2006) and the metabolite profiles of plants are changed (Mewis et al., 2006; Kim and Jander 2007; Kuśnierczyk et al., 2008, 2011) due to application of JA.

The JA functions in plant defense against insects have been described in *Arabidopsis*, tobacco, wheat and sorghum (Kuśnierczyk et al., 2011). The exogenous application of JA or methyl jasmonate (MeJA) induces defense traits (Fritz, 2010). Korth and Thompson (2006) revealed that JA, MeJA and their precursor, OPDA are potent inducers of proteinase inhibitors (PI), and play important roles in plant responses to herbivore attack. At gene level studies, COI1, JAR1,TIR, AXR1, ATMYC2, LOX and MI genes were found to play important roles in JA mediated resistance to insect herbivory in many plants (McConn et al., 1997; Moran and Thompson, 2001; Balbi and Devoto, 2008; Fujita et al., 2009; Morkunas et al., 2011). Several genes viz. COI1, JAR1, TIR1, AXR1, ATMYC2, LOX and MI genes have been found to play important roles in activation of JA and MeJA mediated resistance to herbivory. The expression of these genes activates various sequestering pathways of defensive volatile production in plants which has been reported by many research workers as Moran and Thomson (2001), Voelckel et al. (2004); Zhu-Salzman et al. (2004); Park et al. (2006); Boyko et al. (2006); Gao et al. (2007); Smith and Boyko (2007); Morkunas et al. (2011).

Effect of jasmonic acid on plant attributes

Studies of the last two decades have established the role for jasmonates as signalling molecules or stress modulating compounds (Thompson and Goggin, 2006; Fritz et al., 2010; Kusnierczyk et al., 2011). They have been involved in plant response to wounding and pathogen attack (Farmer and Ryan, 1992; Creelman and Mullet, 1997; Baldwin et al., 1997). Because of their ability to provide protection against biotic and abiotic stresses, jasmonates have been the focus of much attention in recent years (Tsonev et al., 1998; Mackerness et al., 1999; Wilen et al., 1994). The JA treatment has positive and significant effect on pigment accumulation (Poonam et al., 2013). There are some contrary reports which showed that exogenous application

f Me-JA in excised cotyledons of *Cucurbita pepo* inhibited the accumulation of hlorophyll (Ananiev et al., 2004).

The overall stronger stimulatory effect of JA on photosynthetic pigment ccumulation could be due to its stronger effect on the chlorophyll biosynthesis athway especially during earliest stages of greening (Beale et al., 1978). It is also eported that the JA treatment may increase cytokinin concentration which enhanced -aminolevulinic acid either at synthesis level or at its activity level (Poonam et al., 2013). JA application protects membranes from stress damage (Bandurska et al., 2003). It was observed that this protection of cell membrane mediated by JA is dose dependent along with absence or presence of any stress factor (Poonam et al., 2013).

Proline is an amino acid which accumulates in plants under high stress (Chen and Kao, 1993; Gao et al., 2004). It is a stress marker metabolite which protects the plant from harmful consequences of stress induced oxidative damage and cellular integrity (Ali et al., 2007, Poonam et al., 2013). The JA treated stressed and non stressed plants had mixed results on accumulation of proline. In presence of JA, the stimulation of proline content in heavy metal stressed plants reduced (Chen and Kao, 1993; Gao et al., 2004; Jamalomidi et al., 2013). Jang Soo-Won et al. (2008) and Jamalomidi et al. (2013) reported that foliar application of MeJA increased photosynthesis but, reduced proline content in tobacco plants under NaCl stress. In contrary, Ali et al. (2007) found that proline content increased in roots of *Panax ginseng* when treated with MeJA. Exogenous application of JA stimulated protein content in stressed and non stressed plants (Poonam et al., 2013). JA is reported to induce accumulation of jasmonate induced stress proteins (JISP) in rice seedlings (Rakwal and Komatsu, 2001) and their accumulation in peanut seedlings is dose dependent (Kumari et al., 2006). The JISPS are thylakoid-bounded polypeptides (Maslenkova et al., 1992). Most of JA induced polypeptides were identical to one, induced by abscisic acid (ABA) and sodium chloride (NaCl), leading to assumption that exogenously applied jasmonates act as stress agents (Popova et al., 2003).

The application of JA improves as well as retard the growth of plants depending upon chemical analogue and concentration of application. Low concentrations of MeJA increased the growth of *Cynara scolymus* seedlings, but higher concentrations reduced the seedling growth (Closas et al., 2004; Bojorquez-pereznieto et al., 2013). Foliar application of JA led to a significant change in the

plant metabolism (Redman et al., 2001). The growth and yield of MeJA treated garlic plant increased in non-drought conditions, but more effectively under drought conditions (Bideshki and Arvin, 2013).

Tri-trophic plant signalling

The plant defense systems against herbivores are induced through the octadecanoid pathway, which in turn attract natural enemies (predators or parasites) of plant herbivores (Thaler, 1999a). This pathway was inducible by treating plants with JA or by natural herbivory as noted in case of tomato plants under insect herbivory. Jasmonic acid increased the the plant defense induced with parasitism of caterpillar, pests in an agricultural field (Thaler, 1999b; Farha-Rehman et al., 2010; 2012b). The JA and herbivory induced the attraction of carnivores towards herbivores infesting on lima bean plants (Dicke et al., 1999). Lima bean plants damaged by two spotted spider mites (*Tetranychus urticae*) emitted a complex mixture of volatiles. These volatiles attracted carnivorous mites (*Phytoseiulus persimilis*) a specialist predator of spider mites which eliminated entire population of herbivorous spider mite (Dicke et al., 1999). Induction of volatile synthesis by herbivorous spiders in lima bean plants resembled to the effects caused by JA treatment (Dicke et al., 1999).

The inference drawn from experimental findings indicate that direct and indirect plant defenses against herbivores are reduced in jasmonate deficient plants (Thaler et al., 2002; Farha-Rehman et al., 2010; 2012b). Some species of plants only deterred the herbivores while some other plant species also signaled to natural enemies of herbivores as an effective indirect defense strategy. It was also found that damaged wild plants were more attractive to predator mites compared with undamaged wild plants (Thaler et al., 2002; Farha-Rehman et al., 2010, 2012b). In both the cases, JA was reported to be an essential regulatory component for the expression of direct and indirect plant defenses against herbivory (Thaler et al., 2002; Farha-Rehman et al., 2010, 2012b). Van and Dicke (2004) reported that *Arabidopsis thaliana* defend herbivorous insects and mites, through induced volatiles emission upon herbivory. These volatile guided predators or parasites to reach their herbivorous prey, and thus benefitted both the plants and carnivores. Similar pattern of indirect defense has also been noted in case of pine, maize, lima bean etc (Van and Dicke, 2004; Farha-Rehman et al., 2010, 2012b).

Herbivore strategies to plants

The herbivores evolved mechanisms to obtain food from plants despite the evolution of diverse ways of plant defenses. Relationship between herbivores and their host plants often result in reciprocal evolutionary change, called co-evolution (Futuyma and Slatkin. 1983; Walling et al., 2008). Some herbivores co-evolved ways to hijack plant defenses by sequestering these chemicals and using them to protect themselves from predators (Cornell and Hawkins, 2003; Farha-Rehman et al., 2010).

Different types of herbivory affect several plant tissues, besides affecting primary production, translocation and accumulation of photosynthates to varying degrees (Anonymous, 2008; Farha-Rehman et al., 2010). In the ecological context (plant-herbivore-predator interactions), the interdependence of interacting organism on each other is important for their survival in a complex ecosystem (Baalen and Sabelis, 1993; Marrow et al., 1996). The size of herbivores vary from tiny aphids to insects and mammals of very large sizes (Futuyma, and Slatkin, 1983; Farha-Rehman et al., 2010). Insects are the primary herbivores in many ecosystems and feed on a vast variety of plant species ranging from algae to angiosperms. Insects have been the most significant herbivores and the evolution of land plants switched the co-evolution of insects (Farha-Rehman et al., 2010). Herbivory affects a variety of ecosystem properties primarily through differential changes in survival, productivity and growth form of plant species (Anonymous, 2008; Farha-Rehman et al., 2010).

Aphid saliva: Composition and effect

As compared with grazing insects, aphid results in minimal wounding damage to the leaves for they have unique salivary composition (Vickers, 2011). Aphids produce two types of saliva; the gelling saliva is viscous and contains complex carbohydrates, phospholipids and proteins that appear to be consistently represented in the sheath saliva of a number of aphid species (Cherqui and Tjallingii, 2000). The gelling saliva is secreted when the stylet is penetrated into host tissue and forms a tight sheath around the stylet as it traverses the plant tissue (Cherqui and Tjallingii, 2000). This sealing effect of the gel sheath minimizes any counter reaction from the plant cells of the host (Tjallingii, 2006; Giordanengo et al., 2010). A second saliva component is watery. It is produced by aphids containing a variety of hydrolytic enzymes like pectinases, cellulases, polyphenoloxidases, glucose oxidase and

peroxidases (Miles, 1999). The aphid stylet occasionally punctures a few cells while inserting to the sieve element and in this process pours a small amount of the watery saliva into the cells; concomitantly the insect ingests a mixture of the saliva and cytoplasmic contents of host cell (Martin et al., 1997). This initial sample of cytoplasm and saliva mixture, allows the aphid to judge suitability of sap and make a decision on whether to continue feeding on the host (Tjallingii and Esch, 1993; Powell et al., 2006). The watery saliva is also delivered into the sieve element when the insect is feeding from the sieve element (Powell et al., 2006). The enzymes in the watery saliva collectively help the aphid to repress plant defense responses (Ma et al., 1990; Cherqui and Tjallingii, 2000; Harmel et al., 2008) and prevent sieve tube from occlusion through plugging by callose or any other phloem protein (Knoblauch and Van Bel, 1998; Will and van-Bel, 2005; Giordanengo et al., 2010). The glucose oxidase commonly occurring in the saliva of lepidopteran herbivores has also been identified from the saliva of *Myzus persicae* (Harmel et al., 2008) which is responsible for eliciting local defenses in *Arabidopsis* (De Vos and Jander, 2009).

Simulation of herbivory and signalling

Realistic herbivory simulations in terms of plant responses were noted on combining 50 % leaf area clipping and JA spraying on *Solidago canadensis* rather than either clipping or JA spraying alone (Van Kleunen et al., 2004; Farha-Rehman et al., 2010). Jasmonic acid was a useful plant elicitor for pest management on tomato plants (Thaler, 1999; Farha-Rehman et al., 2010). Mechanical wounding with forceps did not substitute for insect attack on rapeseed and does not mimic well the effects of diamond-back moth herbivory to the plants (Pontoppidan et al., 2005; Farha-Rehman et al., 2010).

Engelberth et al. (2004) reported that some plants protect themselves by airborne signalling against insect herbivore attack. Mechanical damage (simulation herbivory) or natural herbivory induce plant to emit green leaf volatiles including six carbon aldehydes, alcohols, and esters. These volatiles also induced intact undamaged neighboring corn seedlings to rapidly produce JA and emit sesquiterpenes (Engelberth et al., 2004; Farha-Rehman et al., 2010) and to accumulate proline (Farha-Rehman et al., 2010, 2012b). These green leaf volatiles played a key role in plant–plant defense signaling and plant-insect interactions.

Effect of herbivory on plant photosynthesis

On herbivory, the loss of carbon fixation is more due to inhibition in rate of photosynthesis in undamaged leaf tissues rather than on leaf area removal (Zangerl et al., 2002; Nabity et al., 2009; 2013). The removal of only 5 % of the leaf area by caterpillars reduced photosynthesis by 20 % in the remaining foliage of wild parsnip (Zangerl et al., 2002), but equally in oak saplings (Aldea et al., 2006). The magnitude of these effects on photosynthesis, depend in large on the type of feeding damage and the mode of defense deployed by the plant under attack (Nabity et al., 2009).

Arthropods also damage xylem and phloem, which may alter water transport, stomatal aperture, and sucrose transport and thereby reduce photosynthesis in remaining leaf tissue (Welter, 1989). Severing tissue vasculature alters leaf hydraulics and subsequently, nutrient or osmotic transport (Sack and Holbrook, 2006). Insect attack can induce many defense-related responses and concomitantly reduce the expression of photosynthesis-related genes (Kessler and Baldwin, 2002).

The sustained reductions in gas exchange and electron transport increased defense metabolites in wild-type *Nicotiana attennata* plants (Nabity et al., 2013). The suppression in photosynthesis occurred only after sustained defense signaling and mobilization of defense chemicals (Nabity et al., 2012). Down regulation of photosynthesis and simultaneous increases in respiration following herbivory occur in some other plant species synthesizing biocidal defensive compounds e.g. terpenes (Zangerl et al., 2002; Gog et al., 2005). The stomatal conductance (g_s) limits internal CO_2 (C_i) and its assimilation in wild-type plants for days after the initial herbivore attack. The onset of herbivory decreased both g_s and C_i indicating that stomata limit CO_2 assimilation (Nabity et al., 2013), associated with severed vasculature (Sack and Holbrook, 2006).

Schroeder et al. (2001) reported in their experiment that plants control their stomatal apertures in response to various phytohormones and environmental signals to regulate gas exchange and transpirational water loss as well as to defend invasion of microorganisms. The stomatal guard cells regulate gas exchange by altering their shape and aperture (Chen et al., 2012). Precise regulation of leaf gas exchange is essential as it directly affects photosynthesis, transpiration, xylem translocation, and plant water potential (Eisinger, 2012).

Role of glucosinolate-myrosinase system against herbivory

The members of family Brassicaceae are known for accumulating several secondary metabolites, especially glucosinolates following aphid herbivory (Kazana et al., 2007; Ahuja et al., 2010). Glucosinolates are toxic to aphid herbivores and can be hydrolyzed into isothiocyanates (ITCs) compounds to attract natural enemies of herbivores (Kazana et al., 2007; Ahuja et al., 2010). The sulphur containing ITCs are generated on degradation of glucosinolates by the enzymatic reaction of myrosinases and it has biocidal activity (Yan and Chen, 2007). Allyl isothiocyanate (AITC) is one of the degradation product of glucosinolates–myrosinase system and its volatilization help in repelling the attacking herbivores (Lambrix et al., 2001). The synthesis of AITC and other products on herbivory are shown in the following Plate 1.

Exogenously applied AITC induced stomatal closure in *Arabidopsis* via production of reactive oxygen species (ROS) and nitric oxide (NO), and elevation of cytosolic Ca^{2+} (Khokon et al., 2011). Through this mechanism genetic evidences have demonstrated that AITC-induced stomatal closure required MeJA priming. These results suggest that crucifer plants produce ITCs to induce stomatal closure, leading to suppression of water loss and invasion of fungi through stomata (Khokon et al., 2011). ROS and NO are second messengers which play important roles in MeJA and ABA signaling and stomatal closure (Murata et al., 2001; Bright et al., 2006; Munemasa et al., 2007; Islam et al., 2010).

Effect of herbivory on plant growth attributes

In majority of plants, protein, some metabolites and macromolecules (e.g. peptides, enzymes, lignin, phenolic metabolites and cuticular waxes) can serve as defense against herbivores (Gutterman and Chauser–Volfson, 2000). Proline is multifunctional plant metabolite (Szabados and Savoure, 2009). The proline and protein under stress conditions play important roles in plant defense mechanism. The accumulation of free proline is stress marker in a number of plant species subjected to hyperosmotic stress conditions (Oncel et al., 1996; Choudhary et al., 2005; Kavi Kishor et al., 2005, Szabados and Savoure 2009). Proline could play crucial role in phloem feeding elicited water loss therefore induces hyperosmotic and oxidative stress. Studies have proposed that higher accumulation of proline (up to 80 % of amino acid pool) is due to stress (Kohl et al., 1991; Schat et al., 1997, Delauney and

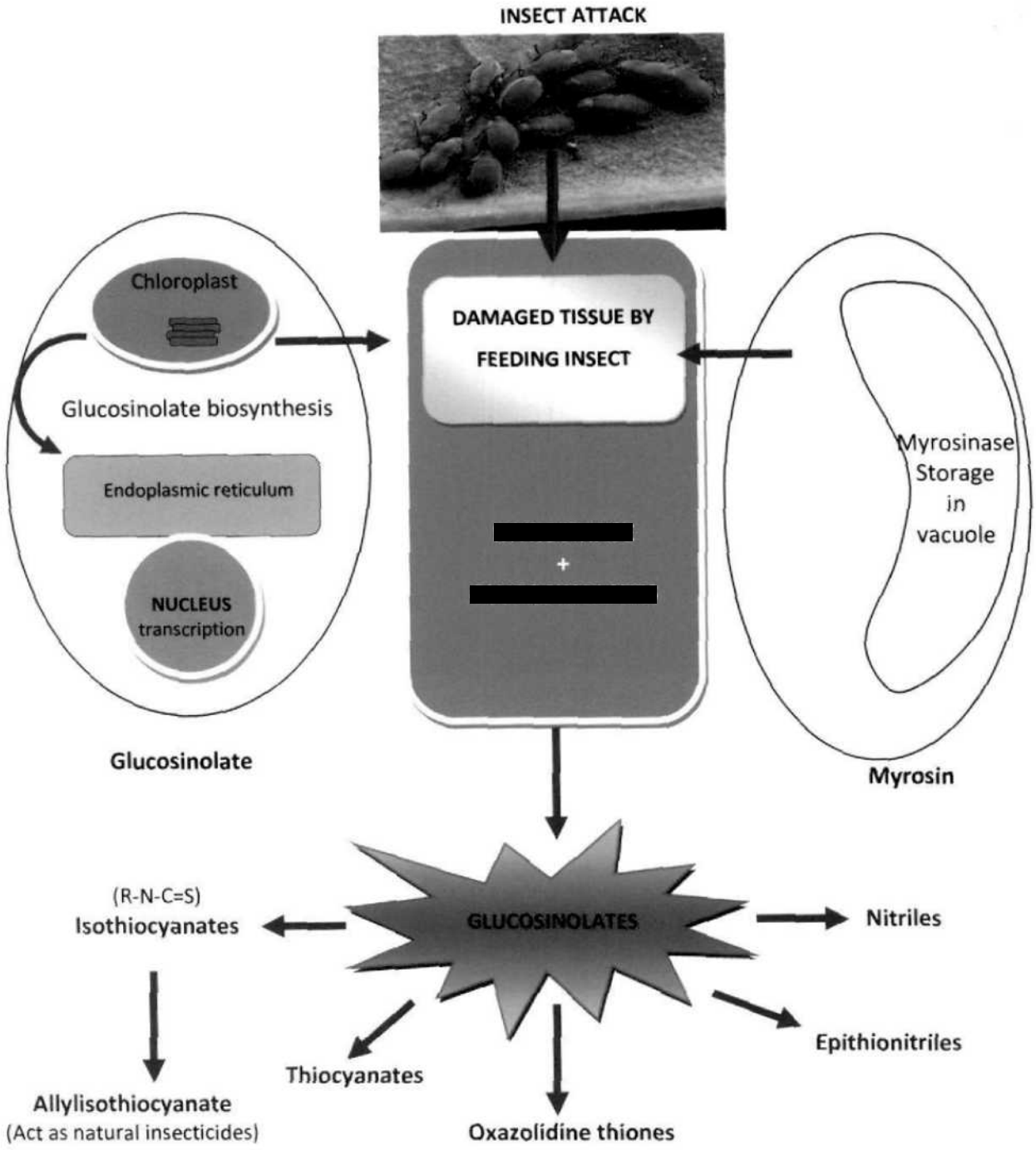


Plate 1. Glucosinolate-myrosinase cycle in mustard (*B. juncea*) activated on herbivory

Verma, 1993; Kavi Kishore et al., 2005), and under normal conditions up to 5% of proline accumulates. Higher accumulation of proline is due to increased synthesis and decreased degradation under various stress conditions proline under osmotic stress conditions; stabilize proteins, cell membranes and subcellular structures (Vanrensburg et al., 1993), and cellular functions by scavenging reactive oxygen species (Bohnert and Shen, 1999). Proline is osmoprotective cellular metabolite (Witt et al., 2012, Szabados and Savoure, 2010; Mewis et al., 2012). Proline was found phagostimulatory to locusts when presented on an inert matrix (Haglund, 1980). Proline (or valine) served as a cue detectable by grasshoppers to lead them to drought-stressed nitrogen-enriched plants (Haglund, 1980). Plants accumulating excessive free proline to resist drought stress may also attract to insect predators (Bright et al., 1982).

Although proline is a universal osmolyte and accumulate in response to several stresses (Oncel et al., 1996), which may have a role in plant defense reactions (Kuznetsov and Shevyakova, 1997), protein degradation is sometimes assumed to be one of the possible sources for proline accumulation (An et al., 2013). The insect attack led to a proportionate loss of leaf protein besides increase in proline accumulation in damaged *Zea mays* leaves (Farha-Rehman et al., 2008, 2012b). The total soluble protein decreased in infested eucalyptus leaves indicating impaired protein synthesis (Singla and Grover, 1994). Drain of assimilates towards the insect directly reduce metabolites in plants (Miles, 1989; Khattab, 2007). Some defensive proteins block the actions of proteolytic enzymes from herbivores are found in legumes, tomatoes and other plants and these defensive proteins accumulate in undamaged tissues in some insect infested plants (Ananthakrishnam, 2001).

Aphid feeding results in oxidative stress in cabbage ascorbic acid, proline, phenol peroxidases, oxidases as well as Ca^{2+} and K^{+} help in the defense mechanism of aphid infested cabbage leaves and thereby delay their death (Khattab, 2007). Herbivory generally stimulate excessive accumulation of proline at the cost of carbohydrate as an adaptive mechanism and there was no significant effect on the phenolics (Miles, 1989; Khattab, 2005). Proline is capable of movement between tissues, and serves as a storage compound for carbon and nitrogen and thus protecting cytoplasmic enzymes and cellular structure (Serrano and Gaxiola, 1994; Hare and Cress, 1997; El-Khawas and El-Khawas, 2008). The increased proline level was

reported in aphid infested barley seedlings (Cabrera et al., 1994) and JA applied root suspension cultures (Ali et al., 2007). The total soluble protein reduced in infested cabbage and *Brassica juncea* (Khattab, 2007; Singh and Sinhal, 2011). Moreover, the reduction in total soluble protein in the infested leaves was concomitant with P level which affected protein synthesis. Similar results were reported by (Singla and Grover, 1994; Khattab, 2007) who found that the rate of protein synthesis declines during stress condition.

Effect on chlorophyll and carotenoid content

The aphid feeding induced a senescence-like state in the alfalfa leaf that is characterized by loss of chlorophyll, decreased levels of soluble protein and fatty acids (Dillwith et al., 1991). After infestation of aphid, susceptible sorghum plants down-regulated some chlorophyll component proteins (Zhu-Salzman et al., 2004; Smith and Boyko, 2007). However, resistant wheat and sorghum plants offer respond to aphid attack by increased synthesis of chlorophyll or photosystem component proteins (Salzman, et al., 2005; Smith and Boyko, 2007). Infestation of attack also reduced chlorophyll levels in cereals (Rafi et al., 1996; Heng-Moss et al., 2003, Golawska et al., 2010).

The phloem sucking aphids change the cell pH either on the luminal side of the thylakoid membrane, preventing the formation of zeaxanthin, or on the stroma side where regeneration of violaxanthin takes place. One of two pathways of natural degradation of chlorophyll *a* is the oxidative bleaching pathway. The decline in chlorophyll level might be due to increased production of defensive compounds (Janave, 1997; Khattab, 2007; Golawska et al., 2010).

Herbivory and plant nutrient

Nitrogen (N) in the soil is absorbed by the plant in the form of nitrate and ammonium ions. It is used by plants to synthesize amino acids, proteins and other complex nitrogenous compounds like chlorophyll. Thus it is essential for plant growth and development (John et al., 2004; Chen et al., 2010). Phosphates help in the formation of nucleic acids and high energy phosphate compounds like ATP (Syers et al., 1986; Wilson et al., 2011; Mochiah et al., 2011). Animals depend on plants for nitrogen supply in the form of proteins and amino acids (Wilson et al., 2011).

Several studies have emphasized the interaction of aphids and their host plants, particularly in the context of plant-defensive responses to aphid herbivory (De Vos et al., 2007; Goggin, 2007). The effect of aphid feeding on host N is independent of host species (Wilson et al., 2011). Wilson et al. (2011) found that N content increased significantly in plants colonized by aphid but reduced in heavily colonized plants. The N enrichment in the aphid-colonized host result into increase of host nitrate reductase (NR) activity. Studies of the past indicated that high NR activity under substrate-limiting conditions can result into increased plant N (Mariotti et al., 1982). The entire process of increase in NR activity from herbivory is not clear. It is speculated that the elevated NR activity of aphid-colonized plants results from transfer of aphid saliva consisting of certain specific biochemical or molecular signals into the host with aphid saliva (De Vos et al., 2007; Goggin, 2007). There are some contrary reports in which N content decreased with aphid infestation depending upon plants species and herbivore insect. Singh and Sinhal (2011) reported that a significant decline in N content was found in *B. juncea* infested with two aphid species as compared to the respective control. The biology of aphid feeding results in a two-way flux of N between the insect and host plant; aphids extract N from host plants in the form of free amino acids and deliver N into phloem in the form of aphid proteins delivered via watery saliva (Mutti et al., 2006, 2008; Will et al., 2007; Wilson et al., 2011).

The reduction of phosphorus (P) in the infested plants may be due to its direct drain through phloem sap by aphid (Khattab, 2007) as was evident from the inverse relationship between aphid population and P content in infested plants (Ridley et al., 2011). However, there was no significant difference in total N content between undamaged and insect-damaged plants. But, N-content in two youngest ramets of *Salvinia molesta* was most vulnerable to insect damage (Forno and Semple, 1987). The damage by *Samea multiplicalis* resulted in a significant loss of K from plant tops than roots and whole plant (Forno and Semple, 1987).

The insect damage did not alter the P levels significantly and constituted less than 0.5% of plant dry weight (Forno and Semple, 1987). Potassium leaches readily from mechanical or insect damaged plants (Tukey, 1970; Room and Thomas, 1986) and loss of K in plants damaged by *Cyrtobagous salviniae* corresponded with insect density (Forno and Semple, 1987). There is no evidence that the K loss affect the

interaction of herbivores insects with their host plant (Forno and Semple, 1987, Leitte et al., 2005). But an increase in N content led to decrease insect population (Leitte et al., 2005).

Volatiles

The phloem-feeding insect inhibits floral volatile production (Walling et al., 2008; Parija et al., 2012). The production and emission of volatiles may differ with floral chemistry, mode of herbivores feeding and evolved strategies of hosts and herbivores. Furthermore, the individuals of *Diaeretiella rapae* have feasibly evolved recognition of certain plant volatiles, and got acclimatized to volatiles chemical cues whilst developing and emerging out of the aphid mummy (Pope et al., 2008). Verheggen et al. (2013) recently characterized the volatile cues emitted by turnip plants (*Brassica rapa*) under attack by an *M. persicae* or by the chewing lepidopteran larva *Heliothis virescens* and tested the behavioral responses of *M. persicae* individuals to the odors of undamaged and herbivore-damaged plants singly or in combination, as well as to the odor of crushed conspecifics (simulating predation).

Phenolic compounds

It is yet not always easy to demarcate between constitutive and induced defense related compounds, as constitutively synthesized and stored chemicals may also be synthesized *de novo* as a response to herbivore damage (Ding et al., 2000; Gatehouse, 2002). To add to this complexity, induced metabolic pathways differ when plants are exposed to different types of herbivory and even species of herbivores. The induction of phenylpropanoid metabolism is implied in the accumulation of newly formed phenolic compounds in several plants in response to herbivore damage (Ding et al., 2000, Santiago et al., 2005, 2006). The phenylalanine ammonia lysase (PAL) activity was enhanced on artificial damage of birch leaf but adjacent leaves did not show such change in enzymatic activity (Hartley and Firn, 1989). But, PAL activity was more pronounced in birch leaves on insect damaged and even adjacent undamaged leaves were signaled for increased PAL activity (Hartley and Firn, 1989).

Phenolic compounds are widely distributed in plants and constitutively present prior to insect or mammalian herbivory-induced damage. The roles for phenolic compounds as pre-formed or constitutive defenses against herbivory are well documented in literature (Ding et al., 2000; Mutikainen et al., 2000; Treutter, 2005;

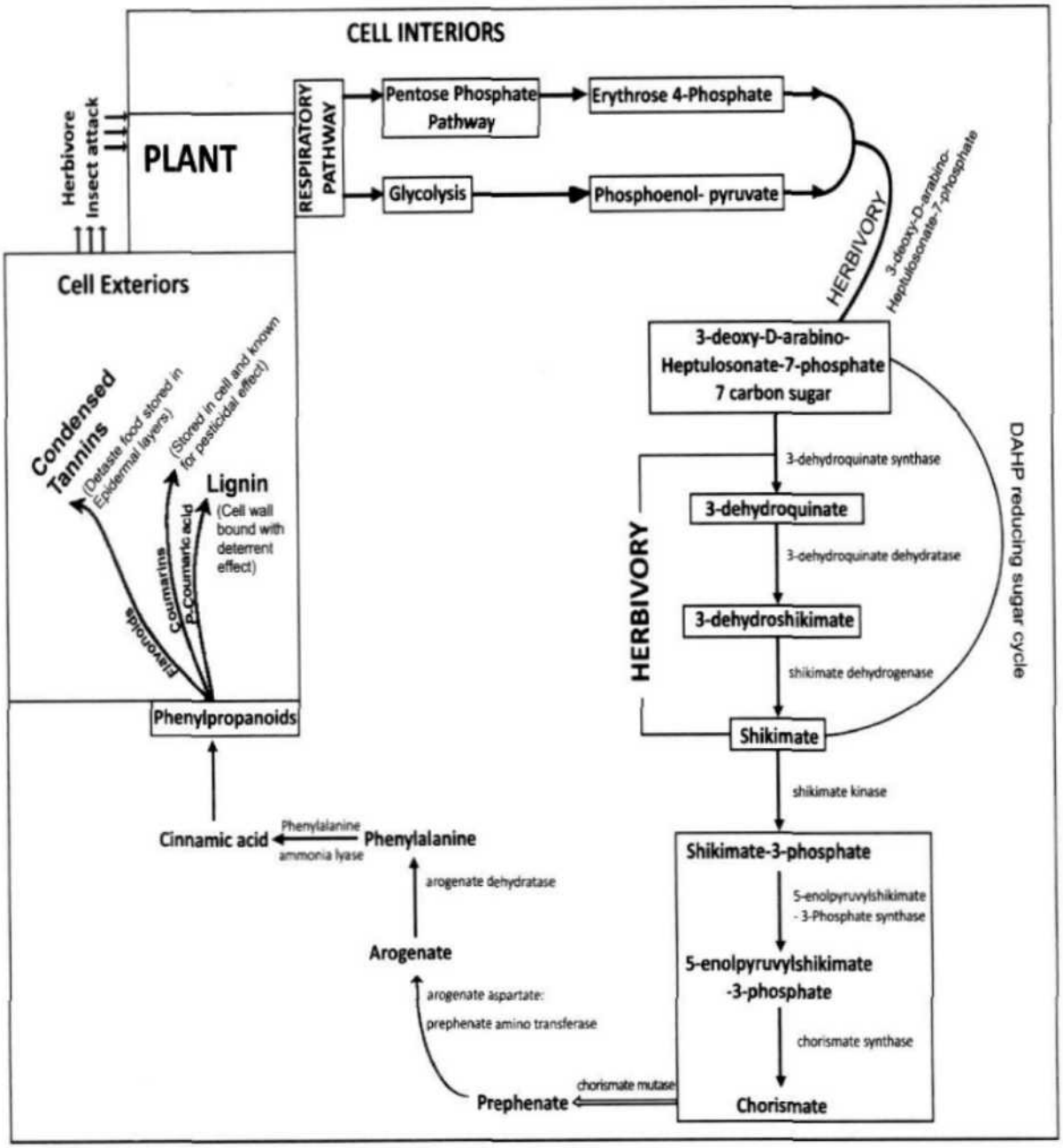


Plate 2: Herbivory induced shikimate pathway and phenyl propanoid pathway facilitate lignin deposition and tannin accumulation in cell wall to deter herbivory.

Santiago et al., 2005, 2006; Farha-Rehman et al., 2012a). It includes cell wall bound phenolics, lignins, suberin, and cuticle associated phenolics as well as stored compounds with a deterring (anti-feedant) or directly toxic (insecticidal) effect on herbivores (Walling, 2000). Phenolics are produced in the leaves of poplar on grazing by gypsy moth larvae, mechanical wounding or treatment with JA (Arnold et al., 2004). Induced defenses are invoked only after tissue damage has occurred and potentially defend host plant at a lower cost of energy in terms of loss of biomass. Thus plants need to balance the allocation of carbon and nitrogen resources between vegetative and reproductive components to ensure survival in the long term (Walling, 2000). The pathway of induced synthesis of phenolic compounds is shown in the Plate 2.

Inferences from the review

In India, mustard (*Brassinca juncea*) suffers a substantial growth and yield loss from aphid infestations. The details of the morpho-physiological growth responses of mustard to direct aphid infestation or its simulations are not well known. The literature review revealed that constitutive and induced defenses in various plant species may deter or kill the herbivore through certain blends of chemicals with deterrent or toxic effects may signal the natural predator of its herbivore. This mechanism is also not well studied and explained. In the present work, an attempt has been made to understand these details.

Chapter 3

Material and Methods

Experiments were conducted on *Brassica juncea* (L) Czern & Coss, grown in the earthen pots, to work out the responses of selected cultivars of mustard plants to the aphid herbivory and chemically simulated herbivory. The plant protective effects were induced using JA, a biochemical mimic of herbivory. A detailed account of the methodology of insect collection, population count, experimental designs and assessment of plant responses are given below under respective heads.

Selection of host plants

Healthy and authentic seeds of five mustard cultivars viz. Alankar, Pusa Jai Kisan, Varuna, Sakha and Rohini were obtained from National Research Centre on Plant Biotechnology (NRCPB) of Indian Agricultural Research Institute (IARI), New Delhi. All the five selected cultivars were screened for their relative sensitivities to aphid infestation. After screening of the cultivars, one most resistant and another most sensitive cultivar was selected for further experimentation.

Selection of the insects

Three most common aphid herbivores identified* and belonging to the order Orthoptera (Aphididae) were collected from the test crop (*Brassica juncea*) from the field. These aphids were *Lipaphis erysimi*, *Brevicoryne brassicae* L. and *Myzus persicae*. One species of aphid was selected for the present study, viz., *Lipaphis erysimi*, for further experimental study. The second species under study was Ladybird; *Coccinella septempunctata* was a predatory beetle at third trophic level. Aphids and beetles were collected from the agriculture field of Department of Agriculture, Aligarh Muslim University, Aligarh, India.

Climatic conditions of Aligarh

Aligarh has an area of about 5,024 sq kms. situated at 27°52'N latitude, 78°51'E longitude and 187.45 m altitude above sea level. Severe hot dry summers and intense cold winters prevail during the year. The winter extends from the mid of October to the end of March. The monthly average temperature of January, the

* Aphid species were identified by Dr. Equbal Ahmad, Associate Professor, P.G. Dept. of Zoology, T. M. Bhagalpur University, Bhagalpur- 812 007. Email: equbal.tmbu@yahoo.com

coldest month, was about 13°C. The minimum temperature for any single day was 5.0°C. The summer extends from April to the end of June and the average temperature for June is about 34°C, whereas the extreme maximum record is 45.5°C. The monsoon extends from the end of June to mid of October. The mean annual rainfall was about 847.3 mm. More than 85% of the total rainfall occurred during June to September. The relative humidity in the winter ranges from 56% to 77% with an average of 66.5%, in of summer, 37% to 49% with an average of 43%; and in monsoon season, between 63% to 73% with an average of 68%.

Preparation of jasmonic acid solution

Jasmonic acid (JA) was obtained from Sigma-Aldrich Chemicals, USA. Stock solution (5 mM) was prepared by dissolving the required quantity (105 mg) of the JA in 5 ml of ethanol, in a 100 ml volumetric flask. Surfactant tween-20 (5 ml) was added to it and final volume was made up to the mark using DDW. The solution was stored in air tight bottle in freezer. The three concentrations of JA (0.5, 1.0, 1.5 mM) were prepared by diluting the above stock solution.

Filling and sterilization of pots

Earthen pots of 25 x 25 cm were autoclaved after filling with garden soil and compost (3:1). The pots were filled each with 4 kg compost mixture soil. Soil texture was sandy loam with pH 7.8 and average NPK level was 98.72, 8.58 and 108.65 mg per kg of soil, respectively.

Sowing of seeds

Authentic seeds of five cultivars (cvs.) of *B. juncea* viz. Alankar, Pusa Jai Kisan, Varuna, Sakha and Rohini were soaked in 1% $HgCl_2$ (mercuric chloride) for 1-2 minutes then washed 2-3 times with distilled water. Five seeds of equal size were sown in each pot. Thinning was done seven days after the germination to leave three plants of almost equal growth and vigor in each pot.

Experimental set-up

For the all the experiments, ten sets (5 treatments with their 5 respective controls) of fine meshed net houses were constructed (Length x Width x Height; 185 cm x 100 cm x 125 cm) supported with iron rods covered from all the sides. The net of one side was provided with 3 ft. long zip to enter for watering the plants and data

recording. The pots inside net houses were arranged in complete randomized design. Each treatment including control had 5 replicate pots. Each replicate pot had three plants. Details of the experimental scheme are shown in the Table 1.

Table 1. Experimental treatment plan during the three consecutive years

Yearly Plan	Experiments	Treatments
I year	**Experiment 1** Screening of relative susceptibility of 5 mustard cultivars.	40 aphids per plant (45 DAS) of all 5 selected mustard cvs. + control plants (5 cvs., 0 aphid)
II year	**Experiment 2** Experiment with screened resistant and sensitive cultivar	0 aphid 50 aphids 100 aphids. 150 aphids
	Experiment 3 Plant response and defense up to 3rd trophic level (Plant-aphid-beetle) in resistant and sensitive cultivar	0 aphid 50 aphids + 2 beetle 100 aphids + 2 beetle 150 aphids + 2 beetle
	Experiment 4 Responses of resistant and sensitive cultivars to JA (simulation of herbivory).	0.0 mM JA 0.5 mM JA 1.0 mM JA 1.5 mM JA
III year	**Experiment 5** Responses of plants to JA (simulation of herbivory) followed by aphid infestation	0.0 mM JA, 0 aphid 0.1 mM JA + 50 aphids 0.1 mM JA + 100 aphids 0.1 mM JA + 150 aphids

Parameters studied

Following parameters were studied:

Plant growth parameters

1. Shoot length (cm)
2. Root length (cm)
3. Plant height (cm)
4. Plant fresh mass (g)
5. Plant dry mass (g)
6. Leaf number per plant
7. Leaf area (cm^2)

Biochemical and physiological parameters

8. Net photosynthetic rate (P_N) and stomatal conductance (g_s)
9. Leaf chlorophyll (a, b and total) content (mg g^{-1} fresh leaf tissue)
10. Leaf carotenoid level content (mg g^{-1} fresh leaf tissue)
11. Proline content (μ mol g^{-1} fresh leaf tissue)
12. Protein content (mg g^{-1} dry plant)
13. Nitrogen content (mg g^{-1} dry plant)
14. Phosphorus content (mg g^{-1} dry plant)
15. Potassium content (mg g^{-1} dry plant)
16. Total phenol content (mg g^{-1} dry plant)

Histological parameters (light microscopy and SEM details)

17. Stomatal density (abaxial, adaxial surface)
18. Relative stomatal closure indices (RSCI)
19. Scanning Electron microscopy (SEM)

Yield characteristics

20. Number of pods per plant
21. Number of seeds per pod
22. Weight of 1000 seeds (g)
23. Seed yield per plant
24. Length of pod (cm)
25. Oil content in seed (mg g^{-1} dry plant)
26. Volatiles estimation (GC-MS)

Insect population count

27. Growth of aphid population (aphid count) per plant
28. Number of beetles attracted per plant

Experiment 1

The first experiment was conducted to work out the relative susceptibility of five selected cultivars of *Brassica juncea* viz. Alankar, Pusa Jai Kisan, Varuna, Sakha and Rohini, exposed to aphid infestation at the rate of 40 aphids per plant at 45 days after sowing (DAS). The seeds of each selected cultivars of *B. juncea* were sown in 50 pots (5 replicates for aphid infested cultivars with 5 control of each). Sets of 5 replicates of aphid infestation and control were maintained randomly in separate net houses. The least aphid population bearing cultivar henceforth is referred as least

aphid susceptible whereas, highest aphid bearing plant cultivar is considered as most susceptible cultivar to aphid infestation. Plants were kept in net house during the entire experimental period in specially designed cages. At 45 DAS each cultivar was infested with 40 aphids per plant. One set of each variety kept in a separate net house without aphid infestation served as control. Sampling was done at 60 and 75 DAS to analyze their comparative responses. Selected parameters from the above list were taken into account.

Experiment 2

Two varieties of *Brassica juncea* cvs. Alankar and Rohini were screened from Experiment 1 as relatively least sensitive and most sensitive cultivars, respectively. After 45 DAS, 4 sets (4 x 5 = 20) were placed in separate set of net houses. Plants were infested with three varying numbers of aphid population (50, 100 or 150 aphids per plant) and one set of each cultivars was maintained without aphids as control. Sampling was done at 60 and 75 DAS to record plant growth characteristics and insect population as in Experiment 1. Physiological, biochemical, yield and other parameters were studied as enlisted above.

Experiment 3

The aphid resistant cultivar; Alankar and aphid susceptible cultivar; Rohini of *B. juncea* were selected for further experimentation (Experiments 2-5). After 45 day stage, each set of pots (4 x 5) of the two cultivars were placed in separate net houses. Two sets of both the cultivars were maintained without insect (0 aphid per plant) and treated as control. The plants (except control) were infested with aphids (*Lipaphis erysimi*) at the rate of 50, 100 or 150 insects per plant, and 5 days later predatory beetle (Ladybird; *Coccinella septempunctata*) were introduced in each of the net house at the rate of 2 beetles per plant. Sampling was done at 60 and 75 DAS to study the different parameters given in Experiment 2.

Experiment 4

Plants on insect attack synthesize JA to ward off or repels attacking insect and provide cues to their predatory insects. To work out as if plants treated with JA mimic insect attack and invite predatory beetle, the 4th experiment was conducted to simulate natural herbivory. The JA was procured from Sigma Aldrich USA.

Plants of each of the selected cultivar (Alankar and Rohini) grown in twenty pots (4 doses x 5 replicate; 45 DAS) were placed in separate net houses and sprayed with JA (0.5, 1.0 and 1.5 mM per plant) in a way that each plant received 2.5 ml JA solution of varying amounts (0, 0.105, 0.210, 0.315 mg JA). Sampling was done at 60 and 75 DAS to observe the effect of JA on plants. One set of plants was exposed in open environment without insect-net at 60 DAS to invite the beetles on treated plants. The number of beetles was counted. The data of plant responses was recorded as in Experiment 2.

Experiment 5

In the 5th experiment effect of pre-infestation application of JA on follow-up aphid infestation was studied. 45 days old plants of the two cultivars i.e. Alankar and Rohini were treated with JA (1.0 mM; selected from the Experiment 4). At 50 DAS i.e. after 5 days of pre-treatment, aphids with varying numbers of 50, 100 and 150 aphids per plant were inoculated in JA treated sets. One replica of treated set (4 x 5 = 20) was kept outside to naturally invite the beetles. Sampling was done at 60 and 75 DAS to observe the combined effect of JA on aphid-infestation of plants. The plants were sampled as in Experiment 2.

Sampling technique of experiments

To study the effect of herbivory and its simulation on growth, biochemical and physiological characteristics of mustard plants, samplings were done at two vegetative stages (60 and 75 DAS) of plants and at harvest (120 DAS). For the determination of growth, one plant from each pot of a set was taken. Single set contained five pots, therefore, each pot served as a replicate. For growth, destructive sampling was done and plants were taken to the laboratory. Photosynthetic measurements were made on intact plants whereas for pigment analyses samples were taken to laboratory. Plants were cut at the base, and pods were plucked and thrashed manually to record the yield aspects. Seeds were cleaned and collected separately from each treatment for the measurements of seed yield and oil content.

Methodology

Plant height (shoots and root length)

For the measurement of root and shoot length, plants were uprooted from the pots. Shoot and root length was measured manually on a meter scale.

Fresh and dry mass of plant

The soil particles from roots were removed by washing. Plants were then wrapped in blotting sheets to absorb the water. Fresh and dry mass of plants were weighed individually using electronic balance. The samples were subsequently transferred in an oven set at 80°C for 72 h. Weight of the dry samples were recorded with the help of an electronic balance (CY204, Scalteo Ins., Germany).

Leaf area

The leaf area was estimated with the help of millimeter graph sheet. One fully expanded third leaf was randomly picked from one third upper part of stem from each replicate. Leaf margin was drawn on graph paper and area was determined by counting directly the cm^2 and mm^2. The data was presented in cm^2.

Physiological and biochemical parameters

Net photosynthetic rate (P_N) and stomatal conductance (g_s)

Net photosynthetic rate and stomatal conductance were measured on fully expanded uppermost leaves of plants using close chamber infra-red gas analyzer (IRGA, LiCOR, 6200, Lincoln, NE, USA) at light saturating intensity between 11:00-12:00h. The capacity of leaf chamber was 1litre. During measurements, the air, relative humidity, photosynthetically active radiation (PAR), ambient temperature and CO_2 concentration were 68±4%, 785±22µmol photons m^2s^{-1}, 24±3°C and 350±5µmol mol^{-1}, respectively.

Total chlorophyll and carotenoid content

Total chlorophyll and carotenoids level in fresh leaves were estimated using the method of Lichtenthaler and Buschmann (2001). One hundred mg of fresh leaves from interveinal area was ground in 10 mL of 80% acetone (Appendix 1) using a mortar and pestle. The suspension was decanted and filtered through a Whatman filter paper No.1 into a Buchner funnel. The optical density (OD) of the solution was read at 645 and 663 nm for chlorophyll estimation and at 480 and 510 nm for carotenoid estimation using a spectrophotometer (UV-1700, Shimadzu, Japan). The chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents were calculated using the following formula.

$$\text{Chlorophyll a} = 12.7\ (A_{663}) - 2.69\ (A_{645}) \times \frac{V}{W \times 1000}$$

$$\text{Chlorophyll a} = 22.9\ (A_{645}) - 4.68\ (A_{663}) \times \frac{V}{W \times 1000}$$

$$\text{Total chlorophyll level} = 20.2\ (OD_{645}) + 8.02\ (OD_{663}) \times \frac{V}{W \times 1000}$$

$$\text{Total carotenoid level} = 7.6\ (OD_{480}) - 1.49\ (OD_{510}) \times \frac{V}{d \times W \times 1000}\ \text{mg g}^{-1}\ \text{FW}$$

Where,

OD = Optical density of the extract at given wavelengths (645, 663, 480 & 510 nm)

V = Final volume of chlorophyll extract in 80 % acetone

W = Fresh weight of leaf tissue (g)

d = Length of light path = 1 cm

Estimation of proline

The proline content was estimated following Bates et al. (1973) in fresh leaves (Appendix 2). 0.5 g of fresh leaf sample was homogenized in a mortar with 5 ml of 3% sulphosalicylic acid. The homogenate was filtered through Whatman filter paper No. 2 and collected in a test tube with two washings each with 5 ml of sulphosalicylic acid. 2 ml each of glacial acetic acid and acid ninhydrin was added to 2 ml of the above extract. This mixture was heated in boiling water for 1 hour. The reaction was terminated by transferring the test tube to ice bath. 4 ml of toluene was mixed to the reaction mixture with vigorous shaking for 20-30 seconds. The chromophore (toluene) layer was aspirated by warming at room temperature. The absorbance of red colour was read at 520 nm against a reagent blank. The amount of proline in the sample was calculated by using a standard curve prepared form pure proline range (0.1-36 µ mole) and expressed as µmoles of proline g^{-1} fresh leaf tissue.

$$\mu \text{ moles of proline g}^{-1} \text{ tissue} = \frac{\mu\text{g proline ml}^{-1} \text{ x ml}^{-1} \text{ toluene}}{115.5} \times \frac{5}{\text{g (sample)}}$$

Where,

115.5 is the molecular mass of the proline.

Preparation of standard curve for proline

50 g of proline was dissolved in DDW 100 ml. From this solution 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml was transferred to different test tubes and the

volume was made to 6 ml using DDW. 5 ml of aqueous sulphosalicylic acid, 2 ml of glacial acetic acid and 2 ml of acid ninhydrin was added. The test tubes were heated on a boiling water bath for 30 min. The reaction was immediately terminated by placing test tube in ice bath. 4 ml of toluene was added in each test tube and after 20-25 seconds red colour appeared. The OD of colour was read it at 520 nm using spectrophotometer. A curve between OD and proline content was plotted for reference.

Estimation of leaf protein content

The protein content was estimated following Lowry et al. (1951) from leaf tissue (Appendix 3). 50 mg of oven dried leaf powder was transferred in glass centrifuge tube, to which 5 ml of 50% trichloroacetic acid was added. The solution was allowed to stand for 30 minutes at room temperature with thorough shaking for the complete precipitation of the proteins. The material was centrifuged at 4000 rpm for 10 minutes and the supernatant was discarded. 5 ml of 1N sodium hydroxide was added to the residue and mixed well. It was left for 30 min on water bath at 80°C to set so that all the precipitated proteins completely dissolved. After cooling for 15 minutes, the mixture was centrifuged at 4000 rpm for 15 min and the supernatant containing protein fraction together with three washings with 1N NaOH was collected in 25 ml volumetric flask. Volume was made up to the mark with 1N NaOH and used for the estimation of proteins. 1 ml sodium hydroxide extract was transferred to 10 ml test tube and 5 ml reagent B was added. The solution was mixed well and allowed to stand for 10 min at room temperature. 0.5 ml folin phenol reagent was added rapidly with immediate mixing. The blue colour developed. The test tube was left for 30 minutes for maximum colour development. Absorbance of the solution was read at 660 nm. A blank containing DDW, reagent B and folin phenol reagent was run simultaneously with each sample. The protein contents were calculated by comparing the optical density of each sample with standard curve plotted by taking known graded dilutions of standard solution of bovine serum albumin

Standard curve for leaf protein content

50 mg bovine serum albumin (Fraction V) was dissolved in 50 ml DDW, of which 10 ml solution was diluted to 50 ml. 1 ml of this solution contained 200 µg proteins. From this 40, 80, 120, 180, 200 µg solutions was transferred to 5 test tubes

separately. The solution in each test tube was diluted to 1 ml with DDW. A blank of 1 ml DDW was also run with each set of determinations, 5 ml reagent B was added to each tube including blank, mixed well and allowed to stand for 10 min. To this solution 0.5 ml folin phenol reagent was added, mixed well and incubated at room temperature in the dark for 30 minute. The optical density of blue colour developed was read at 660 nm.

Digestion of sample for leaf N, P, K estimation

Oven-dried sample (leaf or root) powder (100 mg) was carefully transferred to a digestion tube and 2 ml of concentrated sulfuric acid was added to it. The contents of the flask were heated on a temperature controlled assembly for about 2 h. As a result, the contents of the tube turned black. It was cooled for about 15 min at room temperature and then 0.5 ml 30 % H_2O_2 was added drop by drop and the solution was heated again till the colour of the solution changed from black to light yellow. After further cooling for about 30 min, additional 3 to 4 drops of 30 % H_2O_2 were added, followed by heating for another 15 min. It was repeated till the light yellow colour turned colourless. The digested material was transferred from the tube to a 100 ml volumetric flask with three washings with de-ionized water. The volume of the volumetric flask was then made up to the mark (100 ml) with de-ionized water.

Determination of N, P and K

Determination of N, P and K content in leaf was done in peroxide digested sample (Appendix 4). Nitrogen was determined by the method of Lindner (1944) and phosphorus by the method of Fiske and Subba Row (1925). Potassium was determined using flame photometer Hald (1947). The details of methods are described as under.

Estimation of nitrogen

10 ml aliquot of the digested material was taken in a 50 ml volumetric flask. To this, 2 ml of 2.5 N sodium hydroxides and 1ml of 10% sodium silicate solutions were added to neutralize the excess of acid and to prevent turbidity, respectively. The volume was made up to the mark with de-ionized water. In a 10 ml graduated test tube, 5 ml aliquot of this solution was taken and 0.5 ml Nessler's reagent was added. The final volume (10 ml) was made with de-ionized water. The contents of the test

tubes were allowed to stand for 5 min for maximum colour development. The optical density of the solution was read on a spectrophotometer at 525 nm.

Preparation of standard curve for nitrogen

50 mg ammonium sulphate was dissolved in de-ionized water to get 1 litre solution. From this, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml solutions were taken in ten different test tubes. The solution in each test tube was diluted to 5ml with de-ionized water. In each test tube 0.5 ml of Nessler's reagent was added. After 5 min, the intensity of the colour was read at 525 nm. A blank was run simultaneously with each set of determination. Standard curve of varying concentrations of ammonium sulphate solution versus optical density was plotted and with this help of the standard curve, the amount of nitrogen present in the sample was determined against the OD.

Estimation of phosphorus

The method of Fiske and Subba Row (1925) was adopted for the estimation of phosphorus. A5 ml aliquot of the digested material was taken in 10 ml graduated test tube and 1ml of 2.5% molybdic acid reagent was carefully added followed by the addition of 0.4 ml of 1-amino-2 naphthol-4-sulphonic acid. The colour of this solution turned the colour of the contents blue and the volume was made up to 10 ml. The solution was shaken for 5 min for maximum colour development and transferred to a colorimetric tube. The intensity of the colour was read at 620 nm. A blank was run simultaneously.

Preparation of standard curve for phosphorus

351 mg monobasic dihydrogen orthophosphate dissolved in sufficient de-ionized water to which 10 ml of 10 N H_2SO_4 was added and the final volume was made up to 1 litre. From this, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml solutions were taken in ten different test tubes. The solution in each test tube was diluted to 5 ml. In each test tube, 1 ml molybdic acid reagent and 0.4 ml of 1-amino-2 naphthol-4-sulphonic acid were added and the final volume was made up to 10 ml. After 5 min, the intensity of the colour was read at 620 nm. A standard curve was plotted using different dilutions of potassium dihydrogen orthophosphate solution. The amount of phosphorus present in the sample was determined with this curve.

Estimation of potassium

It was estimated with the help of flame photometer Hald (1947). A 10 ml aliquot was taken and read by using the filter for potassium. A blank was also run side by side with each set of determination. The readings were compared with standard curve plotted using known dilutions of standard potassium chloride solution.

Preparation of standard curve for potassium

Potassium chloride (1.91 g) was dissolved in de-ionized water in 100 ml volumetric flask and volume made to 100 ml. 1 ml of this solution was diluted to 1 litre. This represents solution of 10 ppm potassium concentration. From this 1, 2, 3, 4, 5, 6, 7, 8, 9 and10 ml solutions were transferred to 10 graduated vials separately. The solution in each vial was diluted to 10 ml. The diluted solution of each vial was run separately. A blank was also run with the each set of determination. Standard curve was prepared using different dilutions of potassium chloride solution versus readings on the flame photometer.

Estimation of total phenol

Total phenols could be estimated with the Folin-Ciocalteau reagent using method of Malick and Singh (1980). These hydroxyl containing aromatic compounds react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium and produce blue colored complex (molybdenum blue; Appendix 5). 1.0 g of plant tissue sample was grinded with mortar and pestle in 10 volume of 80 % ethanol. Homogenate was centrifuged at 10,000 rpm for 20 min. Supernatant was saved and residue was re-extracted five times with 80 % ethanol. Pooled supernatant was dried and residue was dissolved in 5 ml of distilled water. Different aliquots of 0.2-2.0 ml were pipetted out into test tubes and volume in each tube was raised to 3 ml. 5 ml of Folin-Ciocalteau reagent was added following 2 ml of 20 % $NaCO_3$ solution after 3 min and mixed thoroughly. Test tubes were kept in boiling water for exactly 1 min and then cooled. Absorbance at 650 nm was measured against a reagent blank. The standard curve was prepared using different concentrations of tannic acid to measure the total phenol in mg phenol per g of dry material.

Stomatal traits

Stomatal density was studied using clear nail polish impressions on leaf epidermis following the method of Teare et al (1971). Thin layer of nail polish was

applied on one side of rib at the middle of the leaf. A small strip of clear cellophane type was gently pressed over the dried nail polish. The tap along with leaf surface impression of nail polish was placed on a slide. The number of stomata were counted under the light microscope on such leaf surface impression of both adaxial and abaxial surface in a cm^2 area of eye piece (= 0.41 mm^2 of leaf surface).

Relative Stomatal Closure Index (RSCI)

RSCI was calculated on adaxial and abaxial surface of leaf with the following formula:

$$RSCI = \frac{Ts - Os}{Ts}$$

Where,

Ts = Number of total stomata

Os = number of open stomata

Scanning Electron Microscopic (SEM) observations

Scanning Electron microscopic observations were preceded by electron microscopic examination of fresh leaf material by scanning electron microscope (JSM 6510 LV JEOL, Japan). A replication method was employed for electron microscopic studies of the leaf surface, since the waxy components are removed by standard fixation and imbedding methods used for ultrastructural examination of cell organelles. The carbon replicas and pseudoreplicas used were prepared from fresh leaf material by a method adopted from Juniper and Bradley (1958) and Whitecross (1963). Ultrasonically treated samples were processed to determine whether the replication technique gives an unaltered picture of waxy surfaces. The electron microscope used was a modified RCA EMU-2.

Yield parameters

Number of pods per plant

At harvest (120 DAS), number of pods in 5 plants of each treatment were counted for the average number of pods per plant.

Number of seeds per pod

From each treatment, 10 pods were randomly selected and counted to get number of seeds in each pod. The average number of seeds per pod was calculated.

Seed yield per plant and 1000 seed mass

The total seeds from a plant in each treatment were cleared, sun-dried and weighed to calculate the seed yield per plant. Thousand seeds were subsequently picked and weighed to record average weight of 1000 seeds of the replicates.

Length of pod

The length of 10 pods, from each treatment was measured on a meter scale for average pod length.

Oil extraction

The oil was extracted by glass Soxhlet apparatus. Five gram crushed seeds of each sample was added to a cellulose thimble in triplicate. Soxhlet apparatus was assembled with the thimbles and a solvent (petroleum ether). The Soxhlet extraction with petroleum ether solvent was performed on the ground seed samples with 30 min of immersion, 45 min of washing and 15 min of recovery at 130 °C.

Preparation for mustard leaf volatile organic compounds (VOCs)

For analysis of mustard VOCs, 5 mL of phosphate buffer solution mixed with active compound in a 22 mL headspace vial and kept air-tight by a PTFE-coated screw cap. A total of 0.25 mL of headspace sample was withdrawn by sampling syringe to inject into the GC column. The automated sampler minimized the variation from human error and improved accuracy and repeatability.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of VOCs

For the evaluation of headspace concentration of VOCs of mustard leaf gas chromatography mass spectrometry GC-MS of Agilent 7890A series (Germany) was used. The system was equipped with split-splitless injector and CTC-PAL auto sampler attached to an apolar HP-5MS (5% phenyl polymethyl siloxane) capillary column (30 m x 0.25 mm i.d. and 0.25 µm film thickness) and fitted to a mass detector. One column was connected to a mass spectrum detector, and the other column to the SCD monitor. This arrangement allowed us to identify VOCs qualitatively and quantitatively. The columns were operated at constant pressure (8 psi), with a heating rate of $5^0C\ min^{-1}$ from 50 to 250^0C. MS was operated in electron ionization mode at 70 eV. GC-MS analysis was carried out at Dept. of Pharmacy, Jamia Hamdard, N. Delhi.

Insect population count

Aphid population count

The whole plant was divided into three parts, (a) vegetative parts (shoots and leaves), (b) flowering parts and (c) fruiting parts (Kaher and Ratul, 1992). The number of aphids counted on each part by a grid and projected value method with the help of magnifying lens. The aphid number on 2.5 cm^2 lengths of 10 places on stem and 10 places of inflorescence was counted and multiplied with the total infected length of stem and inflorescence. Similarly, the aphid count of mid leaf area was multiplied with the total leaf area per plant.

Beetle population count

Beetles were manually counted at the selected growth stage of plant in five plants of each treatment.

Statistical analysis

The data collected was statistically analyzed for two way analysis of variance (ANOVA) in each Experiment. F-value was calculated and the level of significance was determined at $p < 0.05$ using the SPSS statistical program (ver. 12.0 Inc., Chicago, USA). Least significant difference (LSD) was calculated for the significance at $p < 0.05$.

Chapter 4

Experimental Results

RESULTS

In this section, results of five experiments are presented. These experiments were conducted to screen five locally grown cultivars of *Brassica juncea* viz. Alankar, Pusa Jai Kisan, Varuna, Sakha and Rohini, to find out the least and most sensitive cultivars to herbivory caused by aphid *Lipaphis erysimi*. The sensitivity was determined on the basis of relative response of various growth parameters including vegetative growth, physiological and biochemical parameters in addition to population growth of the selected level of aphid population on these cultivars. The screened cultivars (cv. Alankar as least sensitive and cv. Rohini, the most susceptible one) were further tried in the following experiments to study the plant signaling to aphid herbivory and the resulting effects on plant growth. Separate experiments were conducted to find out the effects of exposure to varying numbers of aphids, combined effects of aphid and its predatory beetle, effects of treatments of plant with jasmonic acid (a chemical simulation of natural herbivory) alone or as aphid-pre-infestation treatment to deter attacking aphids and inviting predatory beetles) on plant growth. With selected plant growth parameters, morpho-physio-biochemical changes in two selected cultivars and population growth of aphids and beetles have been studied. The results are presented as under.

Experiment 1

Screening of five mustard cultivars for aphid attack sensitivity

Five commonly grown cultivars of *Brassica juncea* namely, Alankar, Pusa Jai Kisan, Varuna, Sakha and Rohini were selected for the screening experiment. All the five cultivars of *Brassica juncea* were inoculated with 40 aphids per plant on 45 days after sowing (henceforth to be referred as DAS).

Fig. 1-4 shows the aphid population growth, total plant height, shoot length and root length at 60 and 75 DAS. The cultivars of *Brassica juncea* have been arranged in the Fig. 1-4 in accordance with their sensitivity level to aphid population. Among five selected cultivars, the Alankar was relatively more resistant to aphid herbivory as growth in aphid population was comparatively least on it followed by Pusa Jai Kisan, Varuna, Sakha and Rohini (Fig. 1). The reductions in plant growth parameters corresponded with the trends of growth in aphid population from an initial

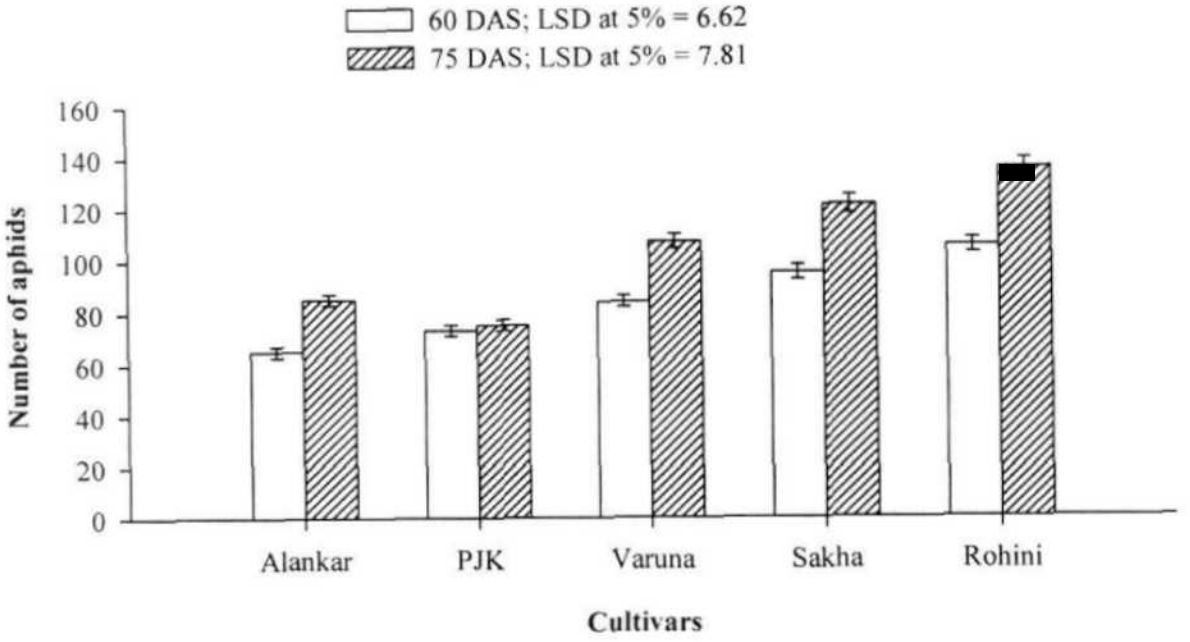
inoculation of 40 aphids per plant on each cultivar. The highest increase of aphid population was noted on *Brassica juncea* cv. Rohini (Fig. 1). The total plant length, shoot length and root length of different cultivars also decreased in accordance with the increase in the population of aphid on selected cultivars at varying growth stages (60 and 75 DAS). The reduction in plant height, shoot length, root length and other growth parameters were linearly correlated with aphid population (Figure LR I and II).

The aphid population increased on all selected cultivars as estimated at 60 and 75 DAS (Fig.1). The cultivar Alankar was relatively least susceptible and cultivar Rohini, the most susceptible one among all selected cultivars. On the basis of the growth of aphid population and corresponding decrease in the overall growth of host plants, all five cultivars screened can be arranged in the following increasing order of sensitivity (from highly resistant to most susceptible cultivar) as Alankar > Pusa Jai Kisan > Varuna > Sakha > Rohini (Fig. 2- 14).

The data summarized in Fig. 5 and 6 shows the effect of inoculation of 40 aphids per plant on leaf number and leaf area of selected cultivars at 60 and 75 DAS. The aphid infestation reduced the leaf emergence and their expansion as evident from the data of leaf number and leaf area per plant in selected cultivars, each inoculated with 40 aphids (Fig. 5, 6). The leaf number and leaf size was badly affected in cultivar Rohini as compared to other (Fig. 5, 6).

The data on fresh and dry mass of five cultivars of *Brassica juncea* infested with 40 aphids at 45 DAS and estimated at 60 and 75 DAS are shown in Fig. 7, 8. The effect on fresh and dry mass of the selected cultivars followed the trend of other growth parameters. The level of variation in dry mass of different cultivars was relatively higher than the variability in other parameters indicating the larger amounts of carbon were partitioned from host to increasing population of herbivorous aphids (Fig. 7, 8). The impact of aphid infestation on plant fresh and dry mass was higher at early stage of plant growth (60 DAS) as compared to late stage (75 DAS) (Fig.7, 8).

The variations in photosynthetic pigments (chlorophyll a, b, total chlorophyll and carotenoids contents) caused by aphid infestation on all the five selected cultivar was also studied and the statistically analyzed data are summarized in Fig. 9-12. The aphid infestation impaired chlorophyll a, b, total chlorophyll and carotenoid contents more



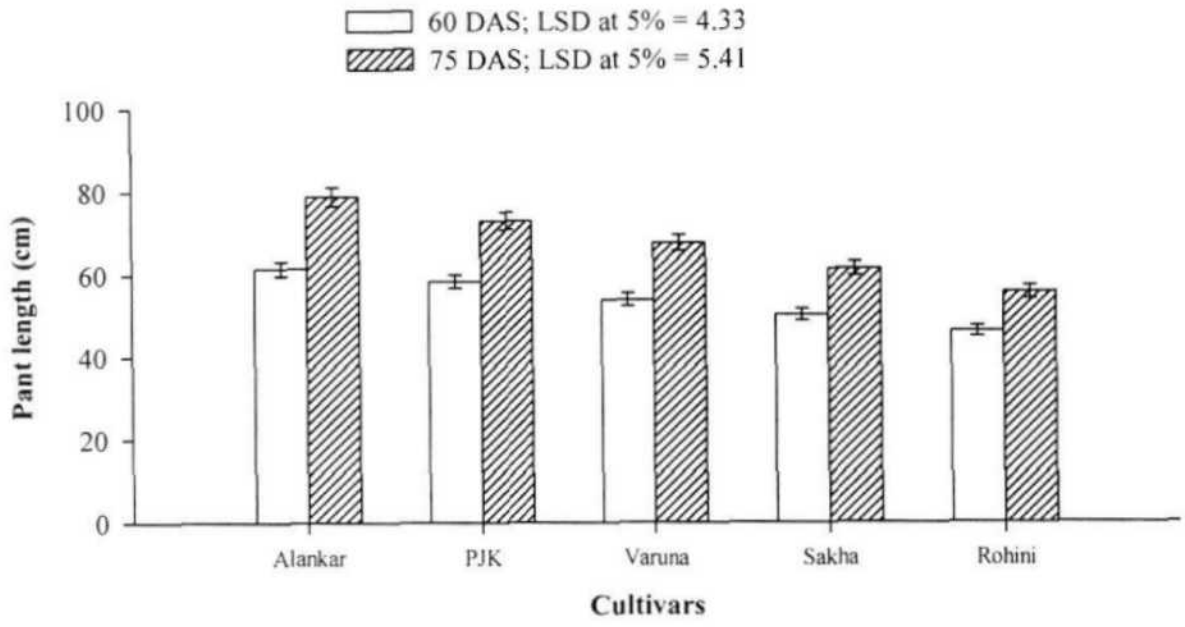


Fig. 1, 2. Effect of aphid infestation (40 aphids per plant) on aphid population growth and plant height (cm) of different cultivars of *Brassica juncea* at 60 and 75 DAS

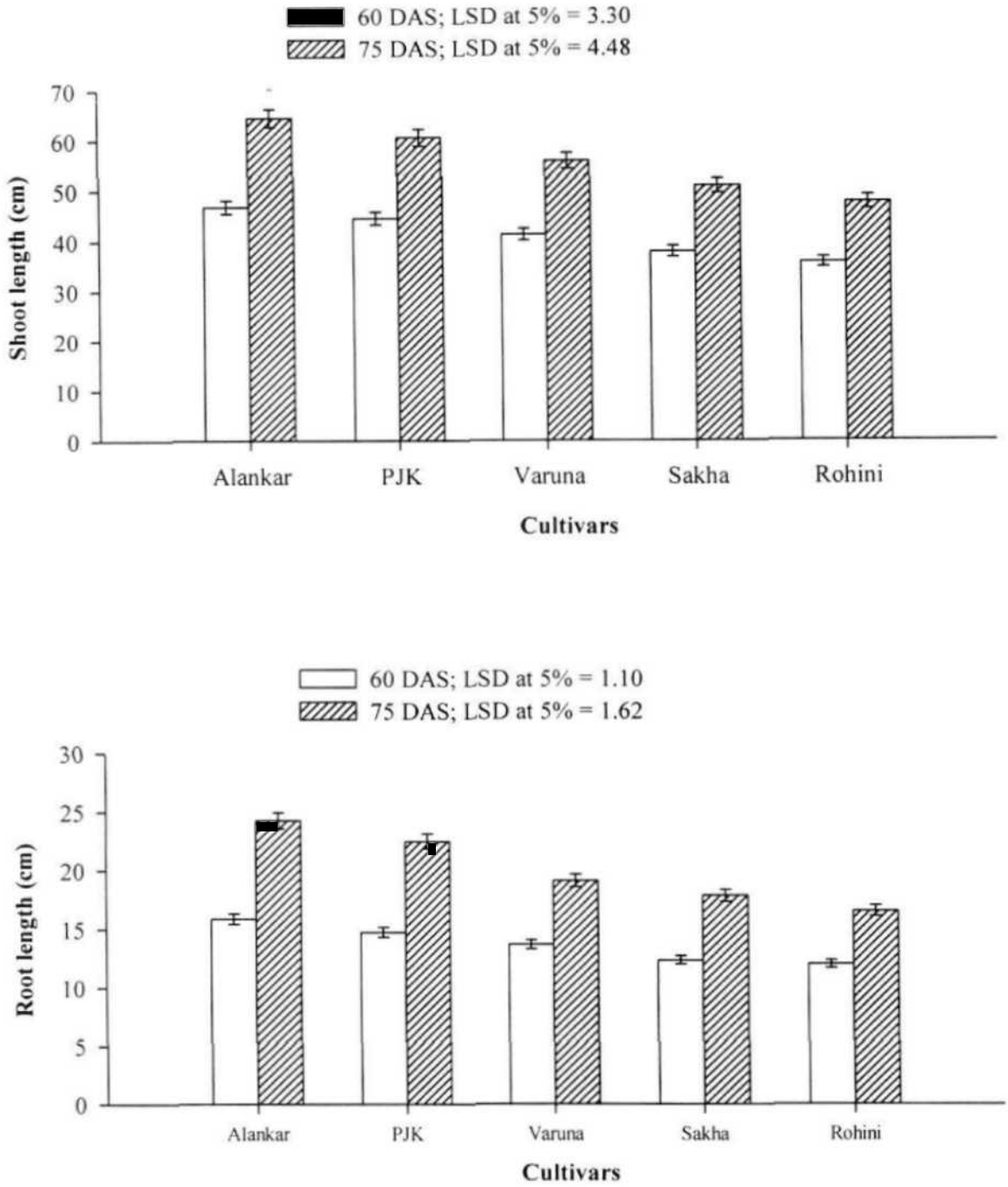
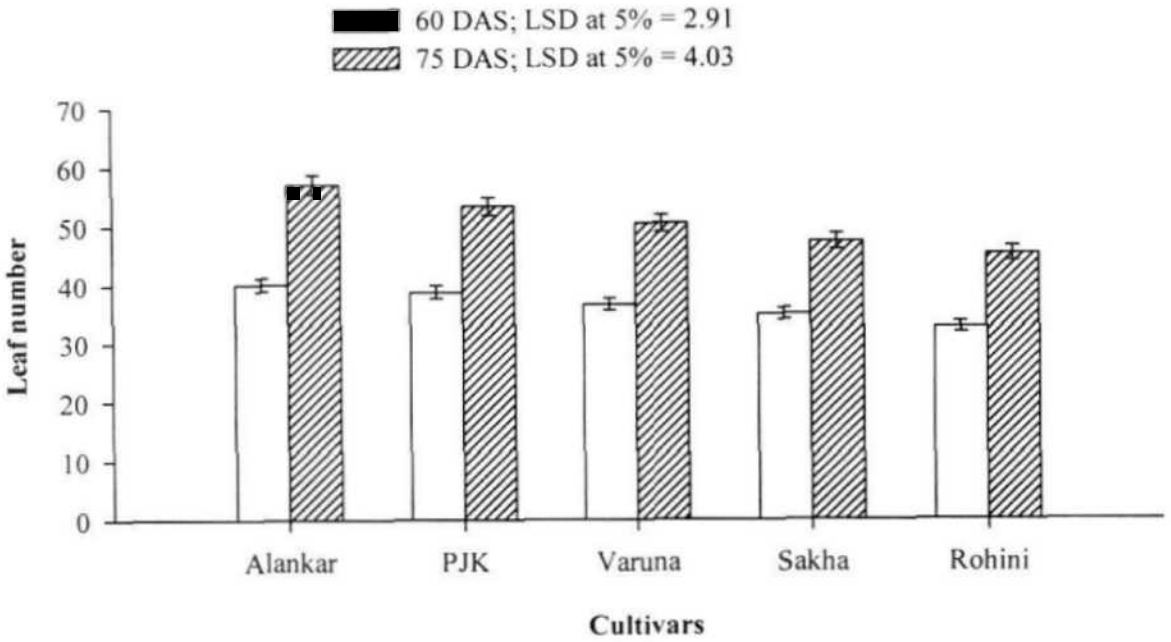


Fig. 3,4. Effect of aphid infestation (40 aphids per plant) on shoot length (cm) and root length (cm) of different cultivars of *Brassica juncea* at 60 and 75 DAS



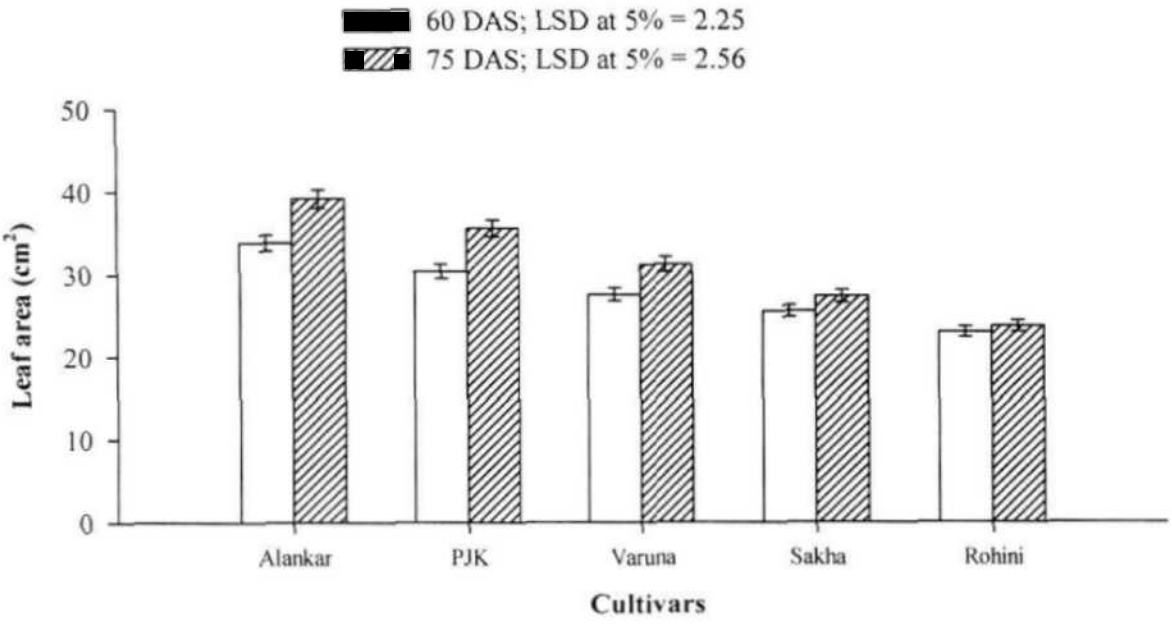


Fig. 5,6. Effect of aphid infestation (40 aphids per plant) on leaf number and leaf area (cm^2) of different cultivars of *Brassica juncea* at 60 and 75 DAS

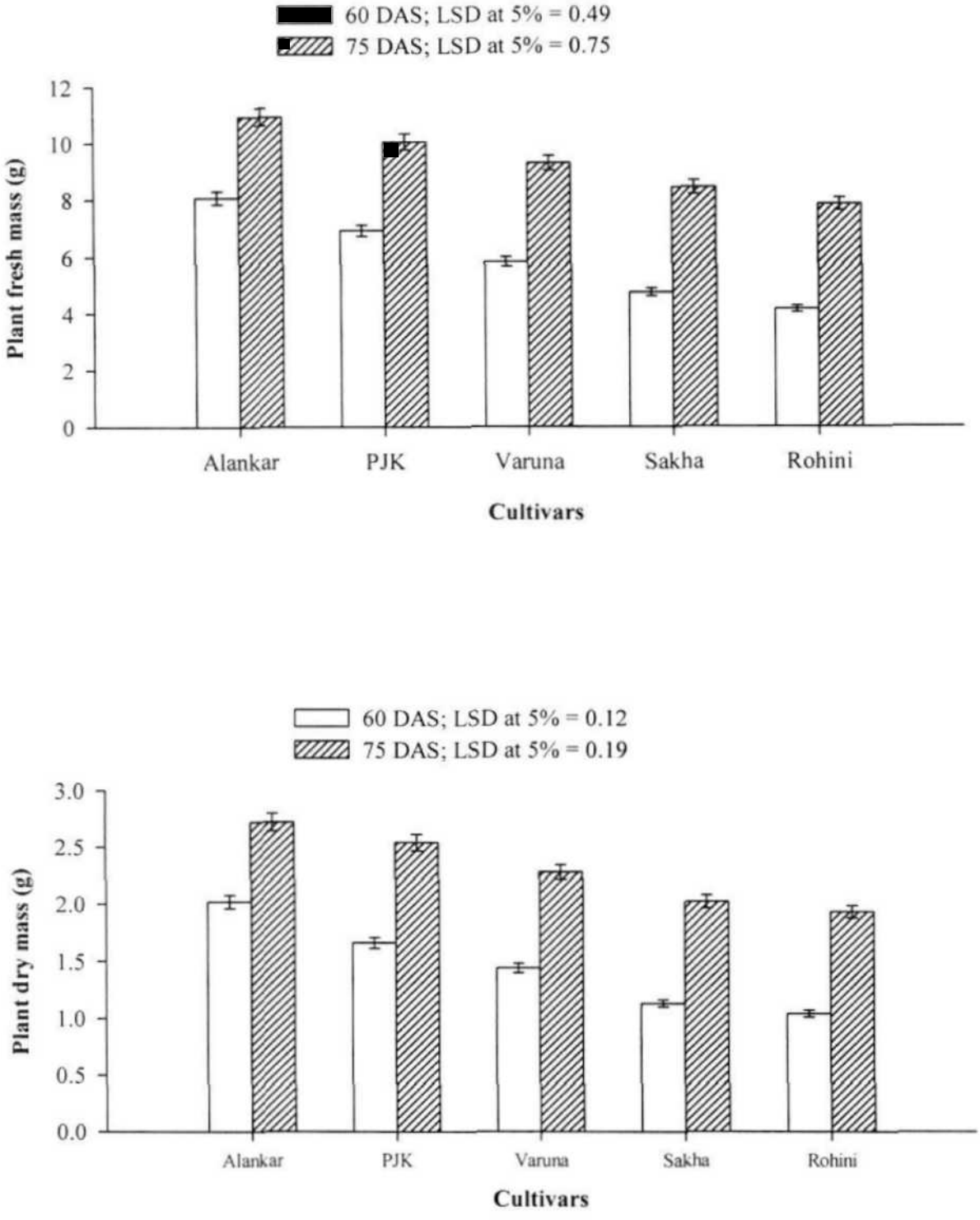


Fig.7,8. Effect of aphid infestation (40 aphids per plant) on plant fresh mass (g) and plant dry mass (g) of different cultivars of *Brassica juncea* at 60 and 75 DAS

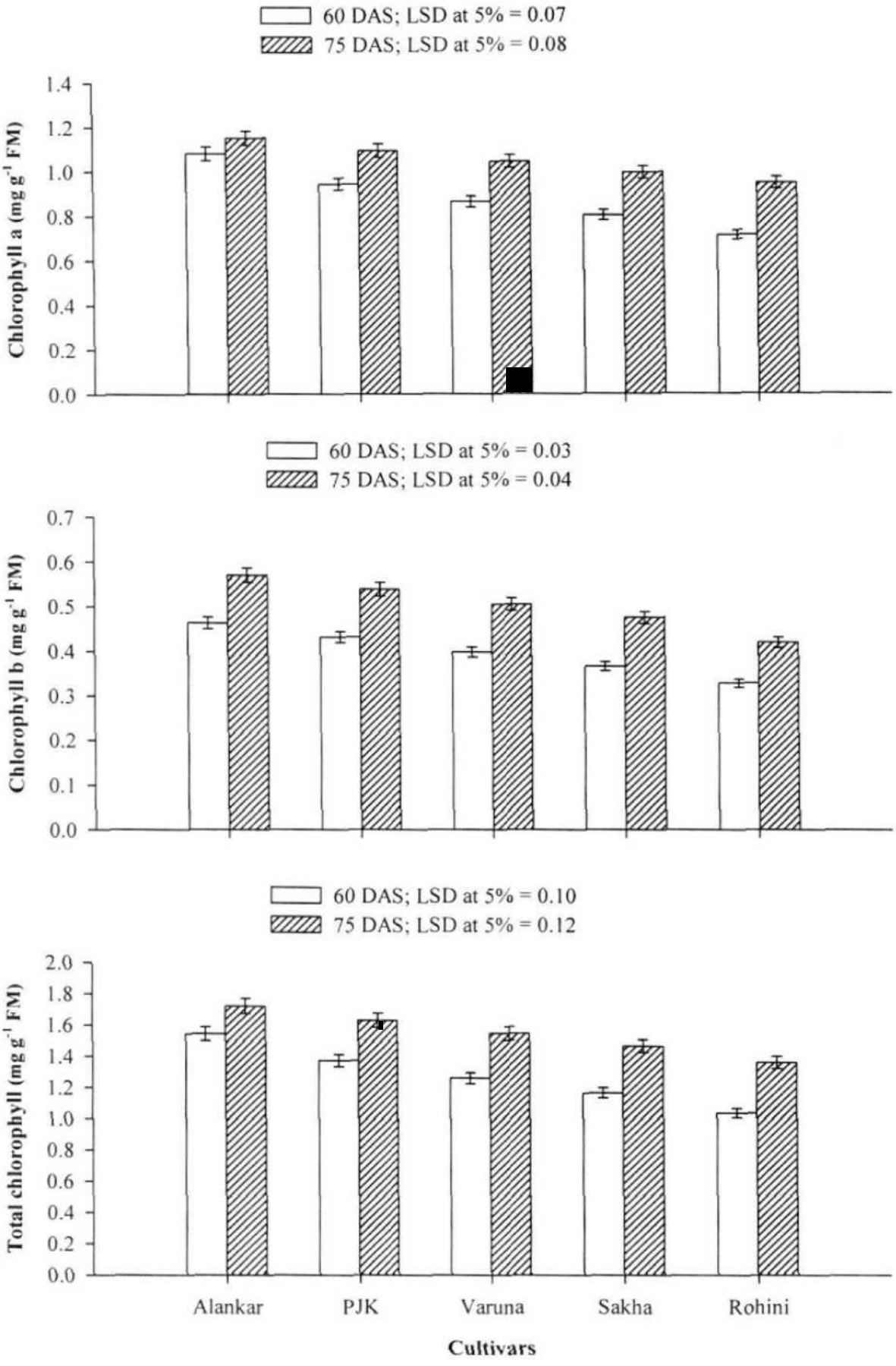
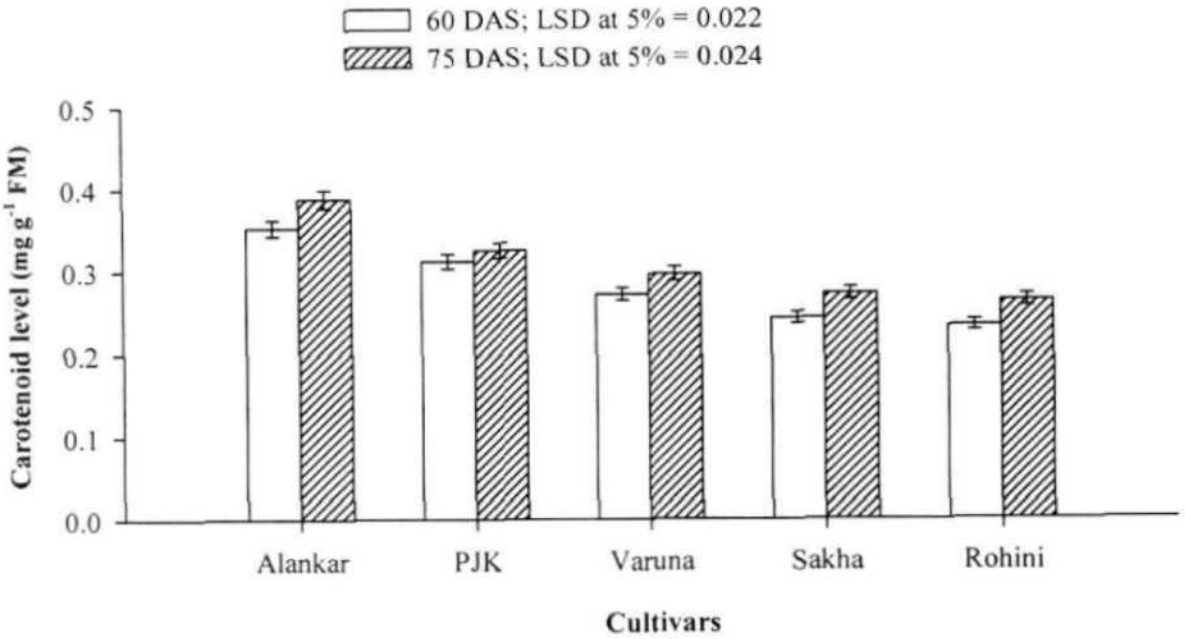


Fig. 9,10,11. Effect of aphid infestation (40 aphids per plant) on chlorophyll a, b and total chlorophyll of different cultivars of *Brassica juncea* at 60 and 75 DAS



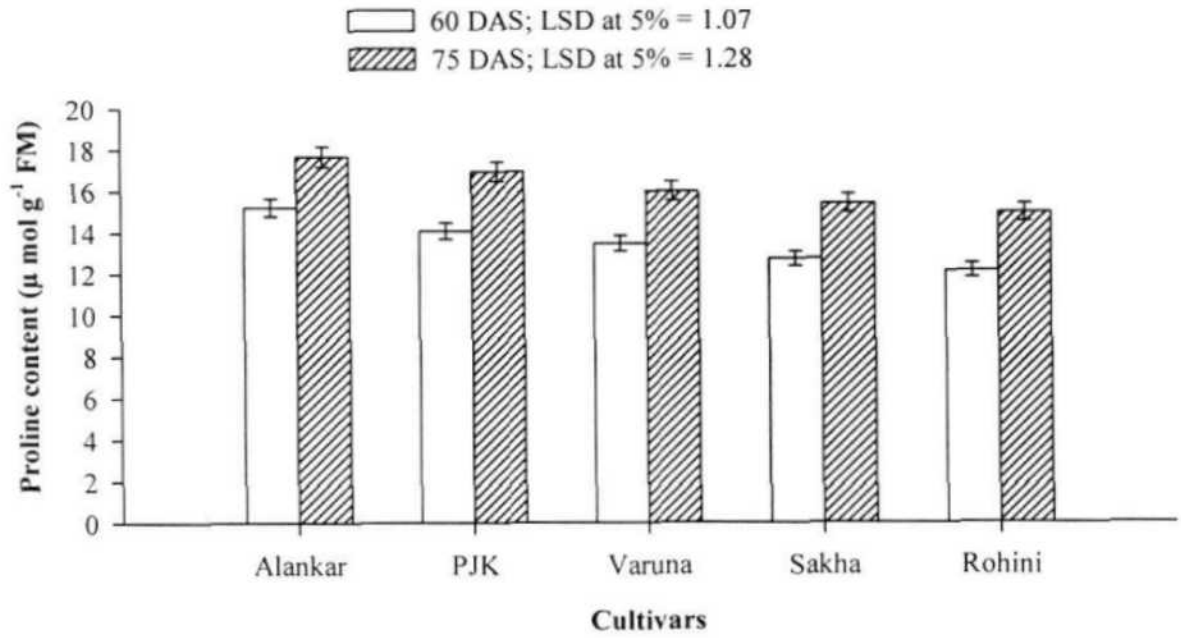


Fig.12, 13. Effect of aphid infestation (40 aphids per plant) on carotenoid (mg g^{-1} FM) and proline content (µ mol g^{-1} FM) of different cultivars of *Brassica juncea* at 60 and 75 DAS

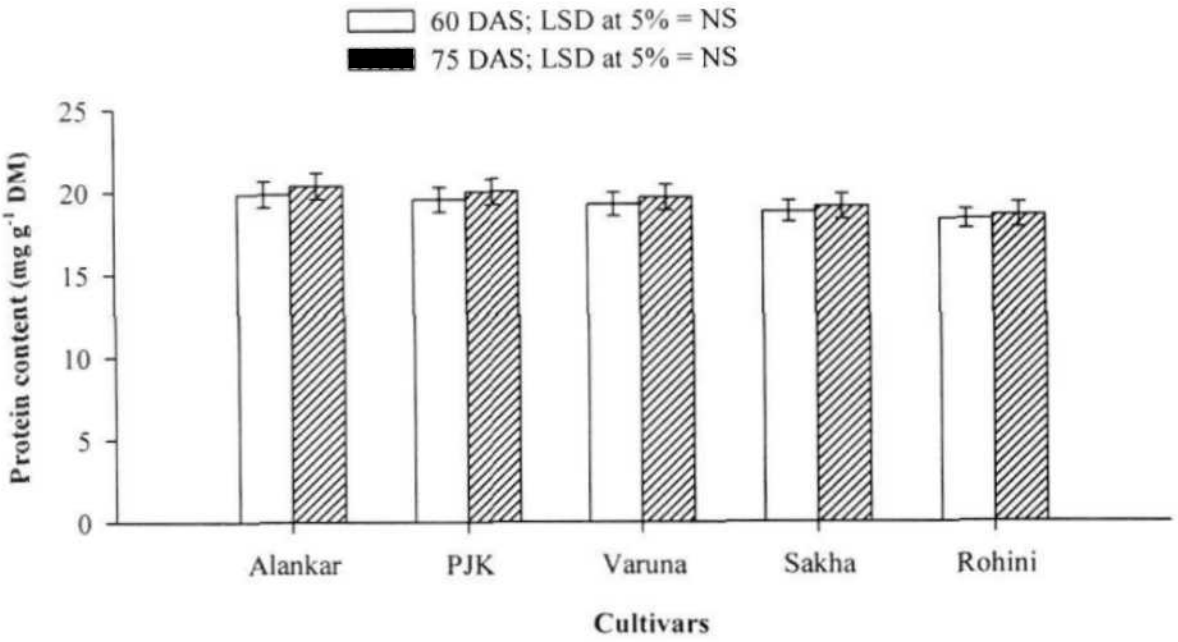


Fig.14. Effect of aphid infestation (40 aphids per plant) on protein content (mg g^{-1} DM) of different cultivars of *Brassica juncea* at 60 and 75 DAS

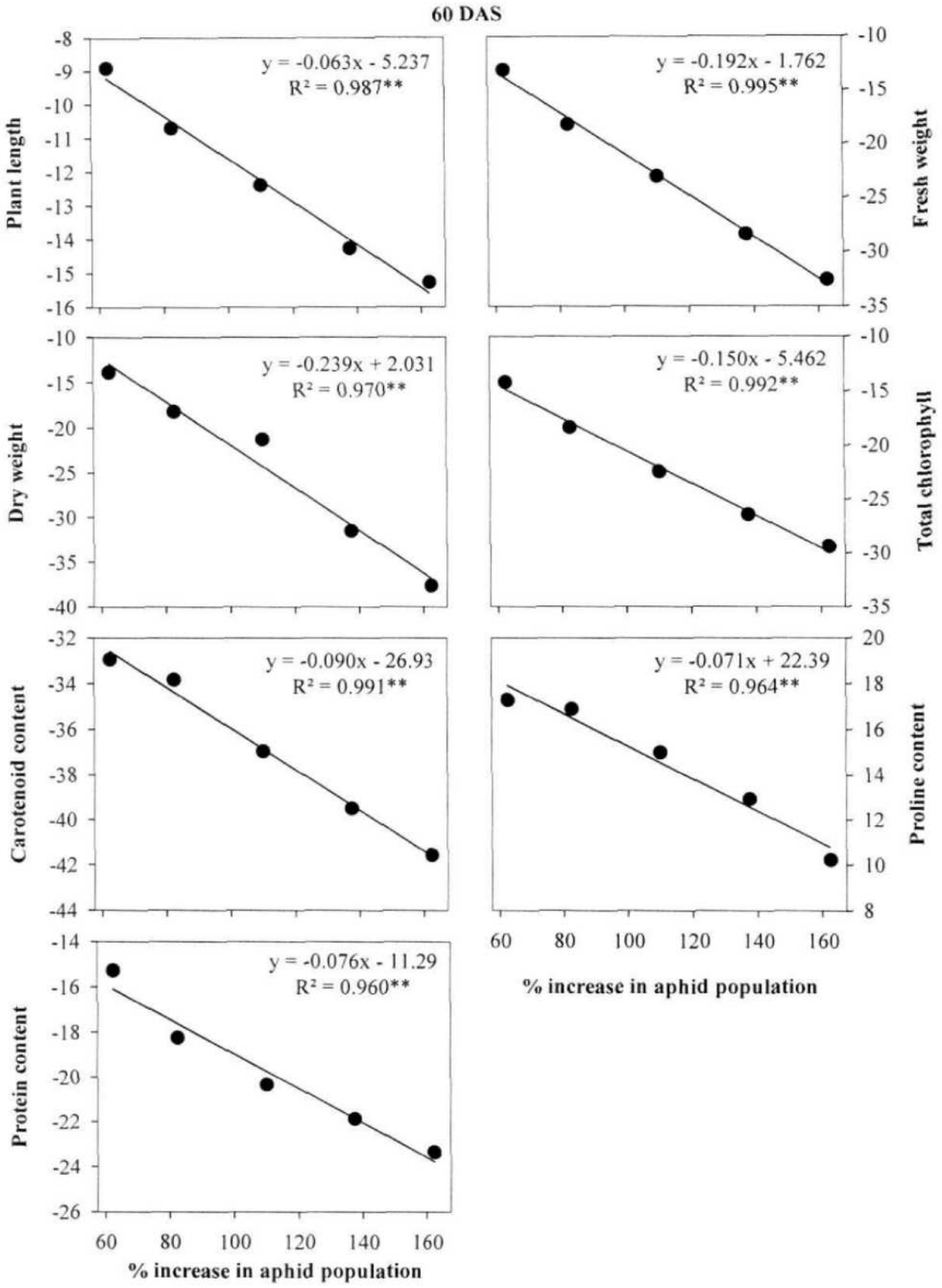


Fig. LR- I. Regression line showing the correlation coefficient between per cent variation in selected growth parameters vs percent increase in aphid population on 5 cvs. namely Alankar, Pusa Jai Kisan, Varuna, Sakha, Rohini, respectively (top to down) at 60 DAS. All selected cultivars were initially infested with 40 aphids per plant and increase in aphid counted at 60 DAS. The percent increase in aphid population on each cultivars treated as independent (X-axis) parameters and percent decrease in various growth parameters dependent (Y-axis).

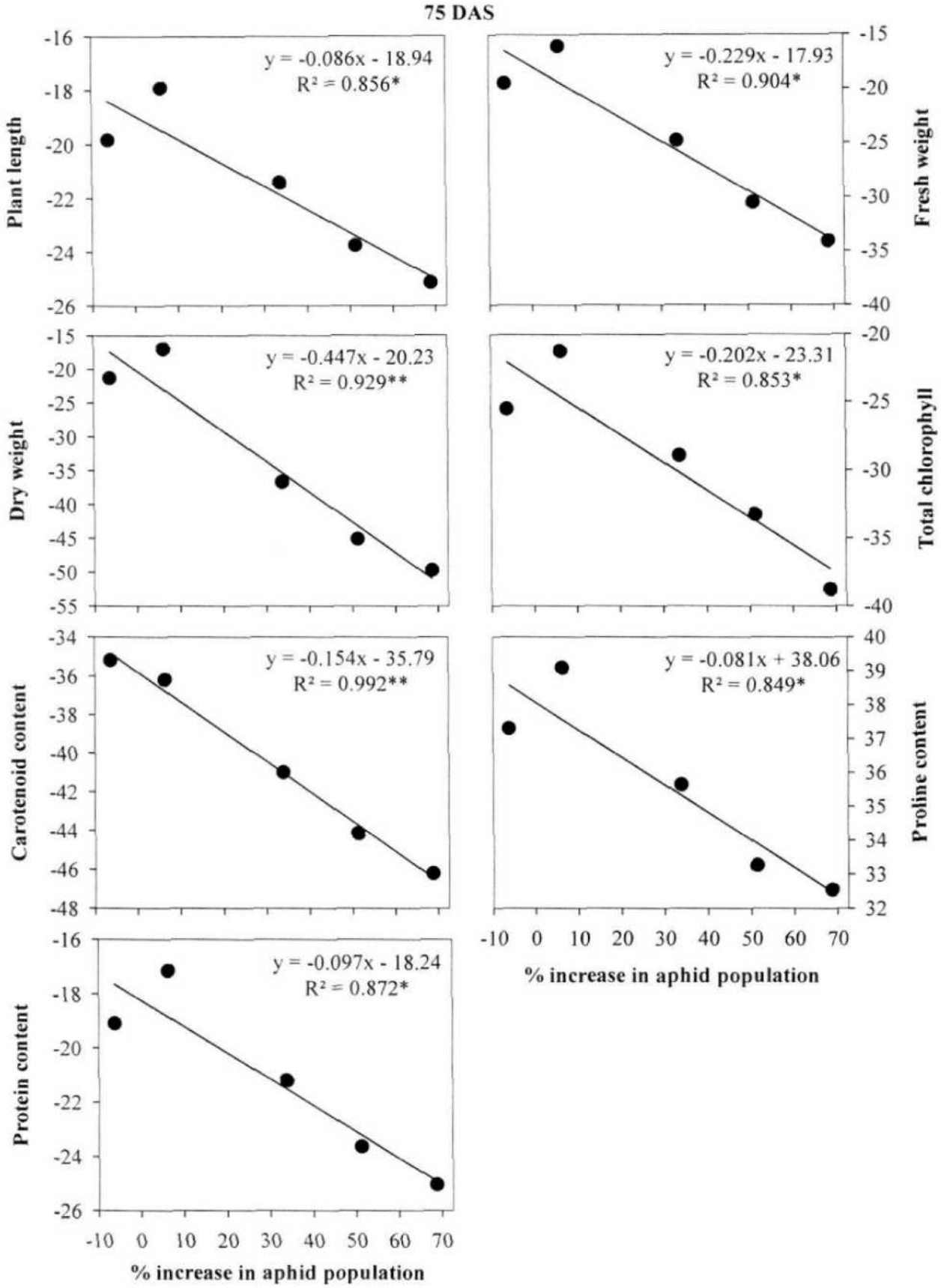


Fig. LR- II. Regression line showing the correlation coefficient between per cent variation in selected growth parameters vs percent increase in aphid population on 5 cvs. namely Alankar, Pusa Jai Kisan, Varuna, Sakha, Rohini respectively (top to down) at 75 DAS. All selected cultivars were initially infested with 40 aphids per plant and increase in aphid counted at 60 DAS. The percent increase in aphid population on each cultivars treated as independent (X-axis) parameters and percent decrease in various growth parameters dependent (Y-axis)

severely in cv. Rohini than cv. Alankar. In all the five cultivars, the loss of chlorophyll a was relatively more severe than the chlorophyll b (Fig. 9-12).

The statistically analysed data of proline and protein estimates of all five selected cultivars are summarized in Fig.13 and 14. It is further evident from the data that among all the five cultivars, Rohini is the most susceptible and Alankar is most resistant to selected aphid species (*Lipaphis erisimi*). The difference in proline content in both the selected cultivars was higher at 60 days growth stage (Fig.13). The protein content was significantly higher in cultivars Alankar than Rohini. There was significant difference in proline and protein content in all the five cultivars (Fig. 13, 14).

Correlation coefficients and regression analysis

The linear regression line between percent increase in aphid population on 5 selected cultivars of *B. juncea* and variations in growth parameter were plotted (Fig-LR-I and LR-II). The square of correlation coefficient indicates that the fresh mass, total chlorophyll and carotenoid contents had strong linear correlations with increase in aphid population on at 60 DAS (Fig. I.R-I). At late stage of growth (75 DAS), the degree of dependence of losses in selected growth parameter over the aphid population reduced to some extent. The cv. Pusa Jai Kisan followed the cv. Alankar in resistance (Fig.- LR II).

Experiment – 2

Impact of varying levels of aphid herbivory

This experiment was conducted to study the comparative response of two selected cultivars of *Brassica juncea*. On the basis of the findings of screening Experiment 1, the least sensitive cultivar of *Brassica juncea* to aphid infestations was Alankar and the most sensitive cultivar was Rohini. These two cultivars were selected for further experiments on growth responses to herbivory, its simulation, related plant defenses and signaling up to third trophic level. The present Experiment 2, was aimed to study the effect of varying levels of aphid herbivory (50, 100 and 150 aphid per plant) on growth and photosynthesis or productivity performances, stress level (proline content) and yield characteristies of Alankar and Rohini having two different sets of inherited defensive traits.

In this Experiment, both the selected cultivars were exposed to varying levels of aphid herbivory (50, 100 and 150 aphid per plant) at 45 DAS. All levels of aphid infestations adversely affected all the growth and biochemical characteristics of both the cultivars, as determined at 60 and 75 DAS growth stages. The effects of aphid herbivory on various plant growth factors were more severe on cultivar Rohini than cultivar Alankar. It emerged from the present experiment that the cultivar Alankar despite being relatively resistant among all five screened cultivars showed reductions in most of the parameters in response to all the levels of aphid population (50, 100, and 150 per plant). But, the losses in almost all growth parameters of cv. Rohini were more severe than in cultivar Alankar. The highest per cent decrease was recorded in shoot and root growth (Fig. 15, 16). The increase in level of aphid herbivory increased the severity of loss in shoot and root length, leaf number and leaf area at 60 and 75 DAS (Fig. 15-18).

The impacts of herbivory by varying populations of aphid on both the selected cultivars of *Brassica juncea* (Alankar and Rohini) were severe on fresh and dry mass of plant (Fig. 19, 20). But, percent reductions were higher in cv. Rohini than cv. Alankar. The impacts of herbivory on all these growth parameters (shoot and root length, fresh and dry mass, leaf number and leaf area) indicates that aphid herbivory caused all these losses more severely due to direct consumption of photosynthates from phloem and resultant alterations in carbon budget (Fig. 15-20).

The chlorophyll a, chlorophyll b, total chlorophyll (mg g^{-1} FM) and carotenoid content (mg g^{-1} FM) reduced significantly in both the cultivars in response to varying level of aphid infestation (Fig. 21-24). The severity in loss of chlorophyll and carotenoid content increased with the level of aphid herbivory (Fig. 21-24). The aphid herbivory reduced total protein and phenol content (Fig. 25, 26) in the infested plants. The aphid feeding increased proline accumulation in both the selected cultivars viz. Alankar and Rohini (Fig. 27). The proline contents were higher in cv. Alankar than in cv. Rohini. The protein content (mg g^{-1} DM) and total phenol content (mg g^{-1} FM) reduced in proportion to the level of aphid herbivory (initially 50,100 and 150 aphid per plant with a proportional increase at 60 and 75 DAS). The impact of aphid infestation on protein and phenol content was more severe at early stage (Fig. 25, 26). The increase in proline content (mg g^{-1} FM) in aphid infested plants was in proportion to the population of aphid (Fig. 27). All levels of aphid infestation (initial number 50,

100 and 150 aphid per plant) also reduced plant nutrients i.e. nitrogen, phosphorus and potassium level (Fig. 28-30). The impact of aphid infestation was more severe on phosphorus and nitrogen content as compared to potassium content (Fig. 28-30).

Visible, microscopic and sub-microscopic symptoms of aphid herbivory

Despite being voracious phloem sap sucker, the aphid did not cause common visible symptoms like chlorosis or necrosis around the infesting area on the leaf unless it transfers a pathogen like fungus or virus causing such symptoms. But on consistently severe and prolonged infestation, the reduction in leaf size, curling of leaf, morphological aberration in floral axis was a common feature in addition to increase in trichome frequency (Plate 3). The hypertrophy in the developing pods was more commonly noted in cultivar Rohini under severe aphid attack. The predatory beetles (*Coccinella septempunctata*) were signaled in larger numbers by cv. Alankar and fewer beetles by cv. Rohini (Plate 3D, E). The aphid attacked plants as part of defensive strategy secreted extra floral nectar (EFN) in the form of fine droplets on leaf surface as a signal to attract mites (Plate 3I). Visual accumulation of a purple-brown pigmenting substance was also frequently seen in the leaves, stem and inflorescence axis around the places of aphid injuries (Plate 3H1, H2, J). The aphid infestation affected the floral axis more severely than the older leaves. The purple-brown saliva accumulated in the epidermal cells and in deeper injured tissues including leaf mesophylls and phloem cells of the leaf veins (Plate 4 A-D). The salivary sheath in injured tissue was formed from gelling saliva of aphid (Plate 4 A). The aphids also damaged cortical parenchyma in the stem and entered its stylet in the phloem cells (Plate 4E, F). The clear damaged cortical cells indicated a cell-sap taste probing site where possibly watery saliva was injected. The aphids, before piercing their stylets into the leaf tissues, damaged the wax layer from outer surface of the leaf cuticle to probe a suitable feeding site (Plate 5C-D). All these features were recorded in both the cultivars (Alankar and Rohini), but these injury features were more severe in cv. Rohini. The aphid attack also damaged guard cells and induced closure of stomata in both the selected cultivars but, more frequently in cv. Rohini than cv. Alankar. Some of the stomata were partially or completely closed (Plate 5A, B).

The aphid infestations severed stomatal frequency, structure and function in injured leaves. But, stomatal aberrations were more frequent in severely injured leaves of cv. Rohini. The frequency of stomata on aphid attack reduced to a relatively

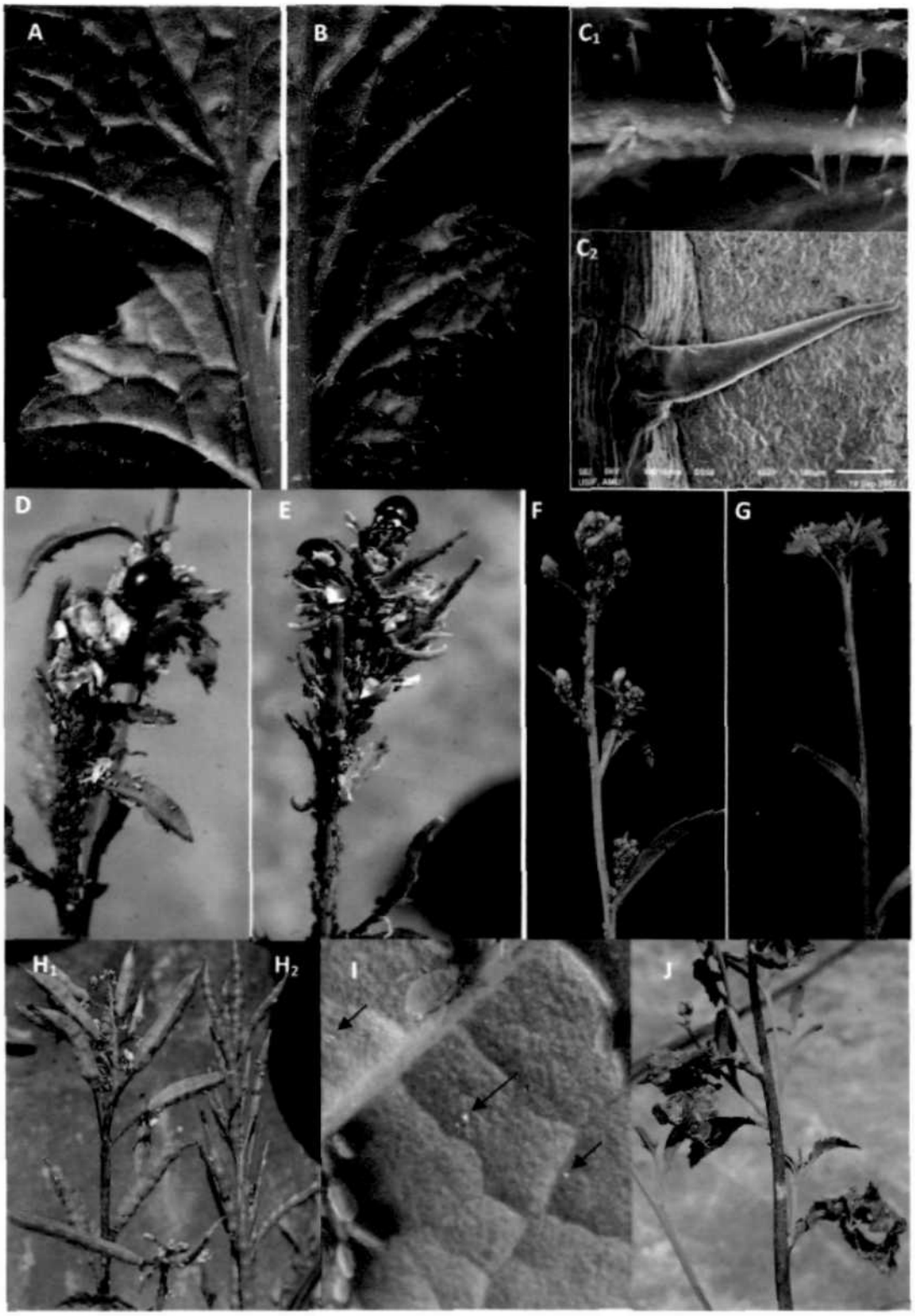


Plate 3. A comparison of morphological features of defense in mustard cvs. Rohini & Alankar. Trichomes at adaxial surface (A) Rohini, (B) Alankar. Zoomed image of trichomes (C_1) light microscopic (C_2) SEM image. Aphid infested during pod formation (D) Rohini (E) Alankar, and early flowering stage (F)Rohini (G) Alankar. Hypertrophied pods (H1) Rohini and (H2) Alankar. Extrafloral nectories (EFN) in aphid infested Alankar (I) and symptoms of aphid infestation (J) purpling, leaf curling & wilting.

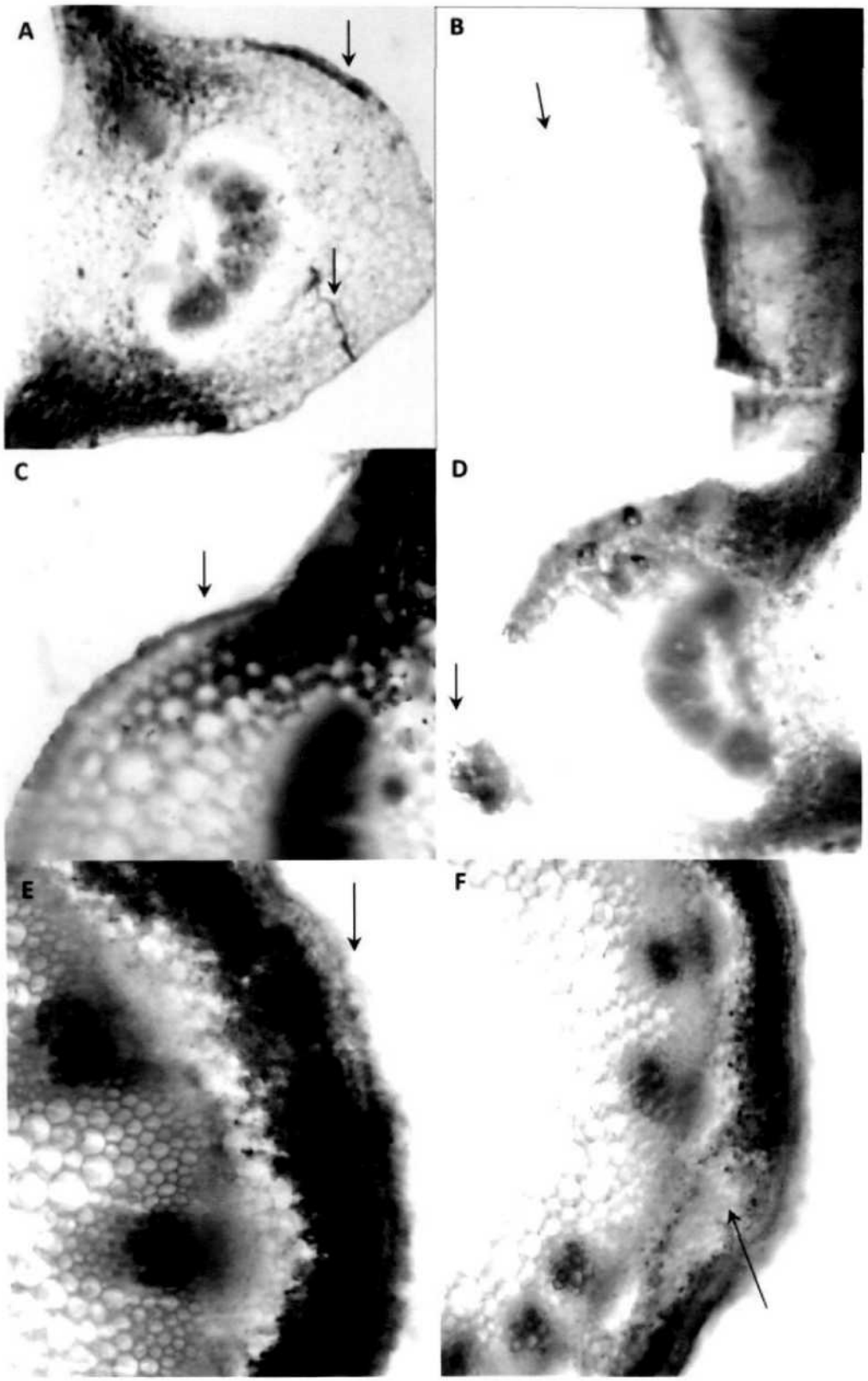


Plate 4. Anatomical effects of aphid infestation on mustard leaf (A) leaf midrib showing aphid salivation and area of stylet insertion, (B) Trichome (C) saliva on cell wall of midrib (D) abnormal growth on midrib (E) Damage at cell-wall of stem section (F) Disturbed cell-wall and underlying cells near salivation area.

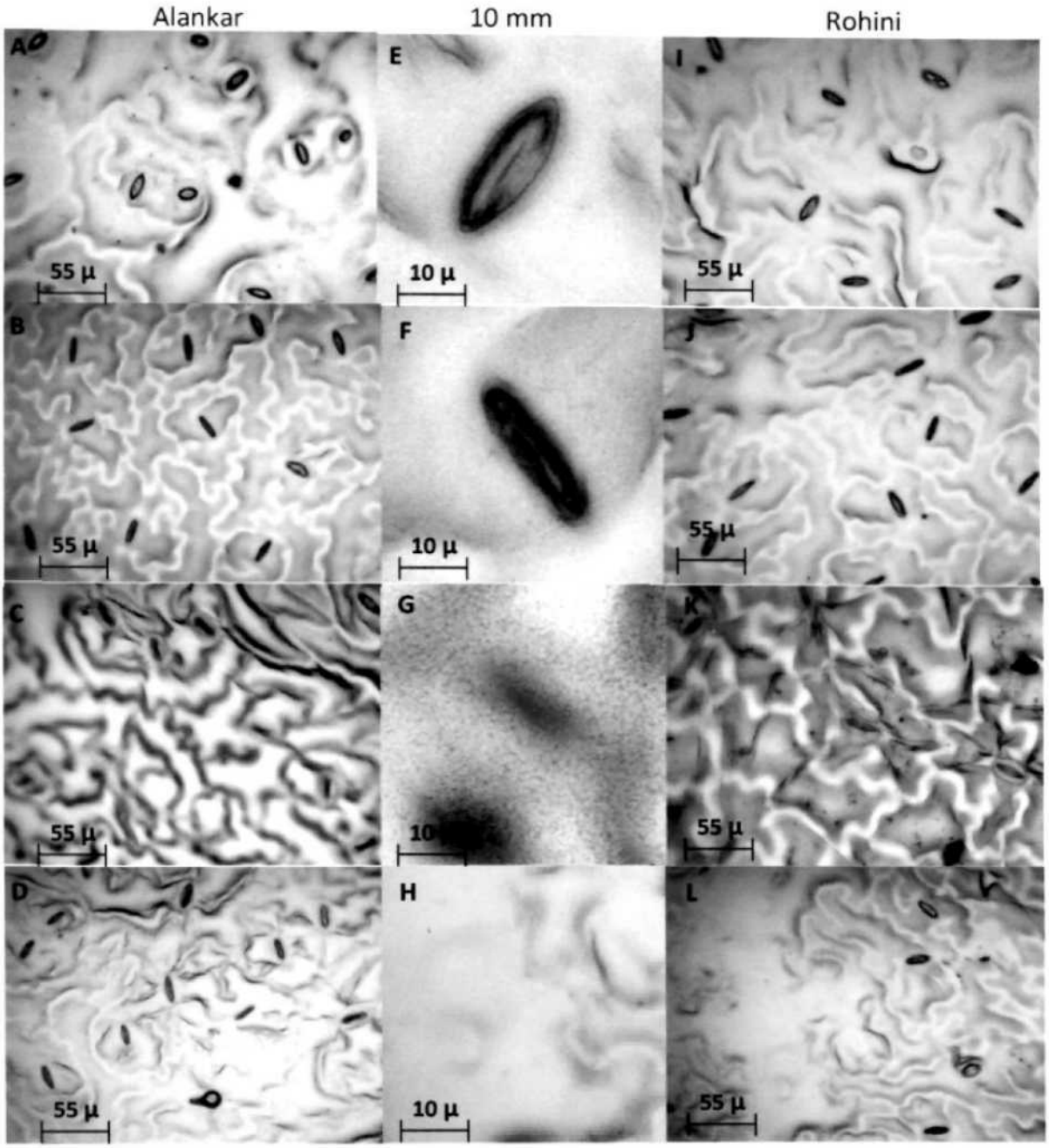


Plate 5. Comparative leaf surface features in cvs. Alankar (A-D) and Rohini (I-L) under light microscope (10×10x, with corresponding scale bars), zoomed light microscopic image of open (E) and closed (F) stomata of common occurrence in both the cultivars, (G) disintegrated guard cells in cv. Rohini infected with 150 aphids, (H) deformed wax layer in cv. Rohini infected with 150 aphids.

larger extent in cv. Rohini than in cv. Alankar (Fig. 31, 32). The level of impact on stomatal frequency corresponded with the population of aphids (Fig. 31, 32) in both the cultivars at 60 and 75 DAS. It is evident from the data of relative stomata closure index (RSCI) that closure of stomata increased with the aphid population (initially 50, 100 and 150 aphid per plant and subsequent increase) in both the cultivars (Fig. 33, 34). But, frequency of stomata closure was relatively larger in cv. Rohini than cv. Alankar (Fig. 33, 34).

The rate of net photosynthesis (in terms of μ mol CO_2 m^{-2} sec^{-1}) and stomatal conductance (in terms of gas exchange mol m^{-2} sec^{-1}) was also recorded in plants exposed to varying level of aphid infestation (50, 100, and 150 aphid per plant). The rate of photosynthesis and stomatal conductance reduced in both the cultivars under varying levels of aphid herbivory (Fig. 35, 36). The rate of photosynthesis and stomatal conductance decreased with the population of infesting aphid and days of infestation (Fig. 35, 36). The reduction in the efficiency of photosynthesis and gas exchange through stomata was higher at the late growth stage i.e. at 75 DAS (Fig. 33, 34).

The aphid infestation had direct impact on the yield of the plant. Substantial reductions were recorded in the pod length of both the cultivars under varying levels of aphid herbivory. The pod length of cv. Rohini was more severely affected in response to all three initial doses of aphid (Fig. 37). The per cent oil content in seeds also reduced significantly on aphid herbivory (Fig 38). The pod and seed setting was adversely affected by aphid herbivory as evident from the statistically analysed data on pod number per plant, seed number per pod and seed outputs per plant (Fig. 39-42). Significant reduction in all yield parameters caused by aphid infestation in both the cultivars Alankar and Rohini reduced oil yield (Fig. 38-42).

Aphid and beetle demography

The selected cultivars (Alankar and Rohini) were exposed to 50, 100 and 150 aphids per plant at 45 DAS growth stage and the increase in aphid population was determined at 60 and 75 DAS. The statistically analysed data are summarized in Fig. 43 and 44. The aphids multiplied more quickly and almost exponentially on both the cultivars. The increase in aphid population was comparatively more on cv. Rohini than on cv. Alankar (Fig. 43, 44). The aphid attacked plants were exposed free to

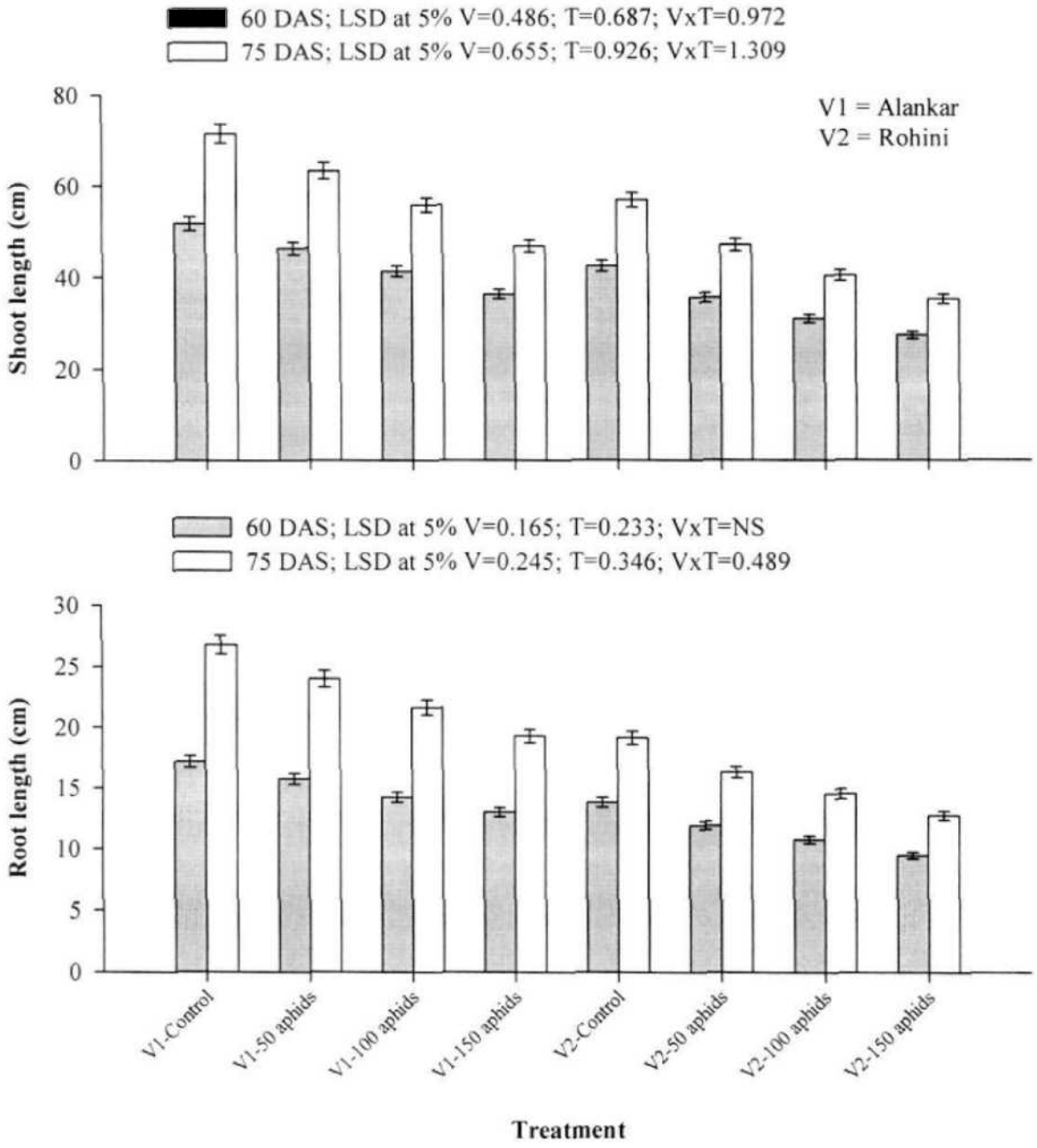


Fig. 15,16. Effect of aphid infestation (0, 50, 100 and 150 per plant) on shoot and root length (cm) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

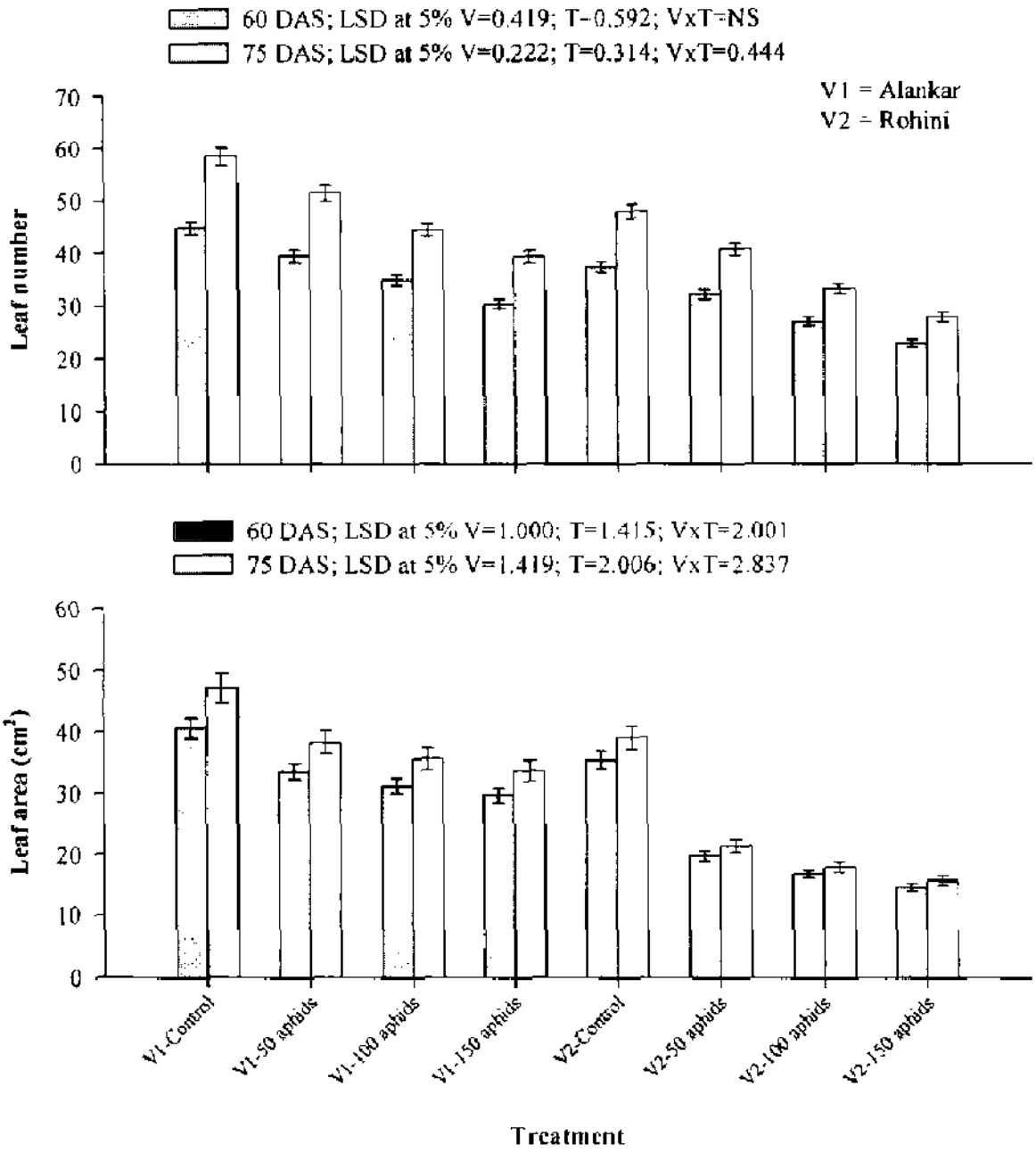


Fig. 17, 18. Effect of aphid infestation (0, 50, 100 and 150 per plant) on leaf number and area (cm^2) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

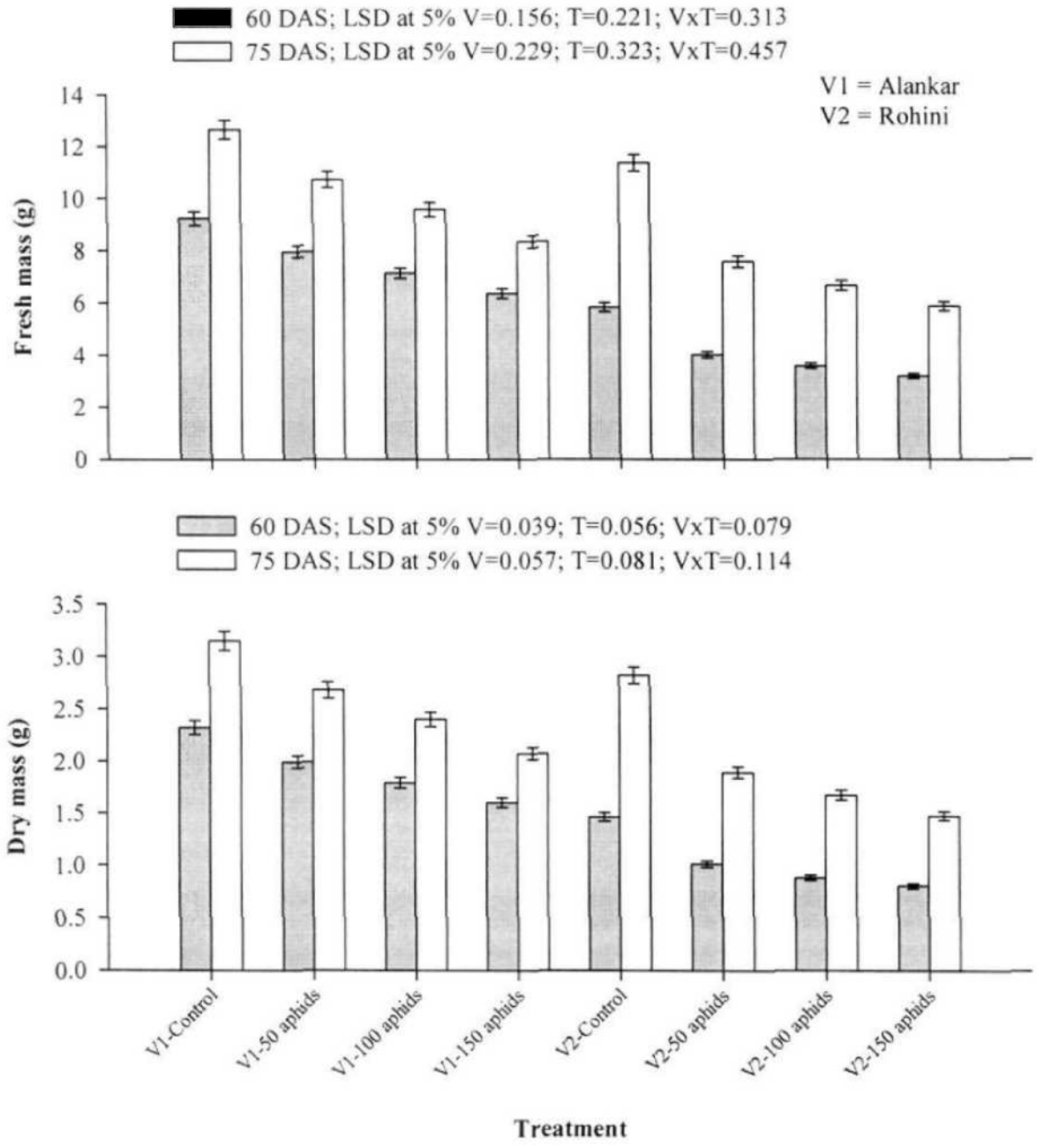


Fig. 19, 20. Effect of aphid infestation (0, 50, 100 and 150 per plant) on fresh and dry mass (g) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

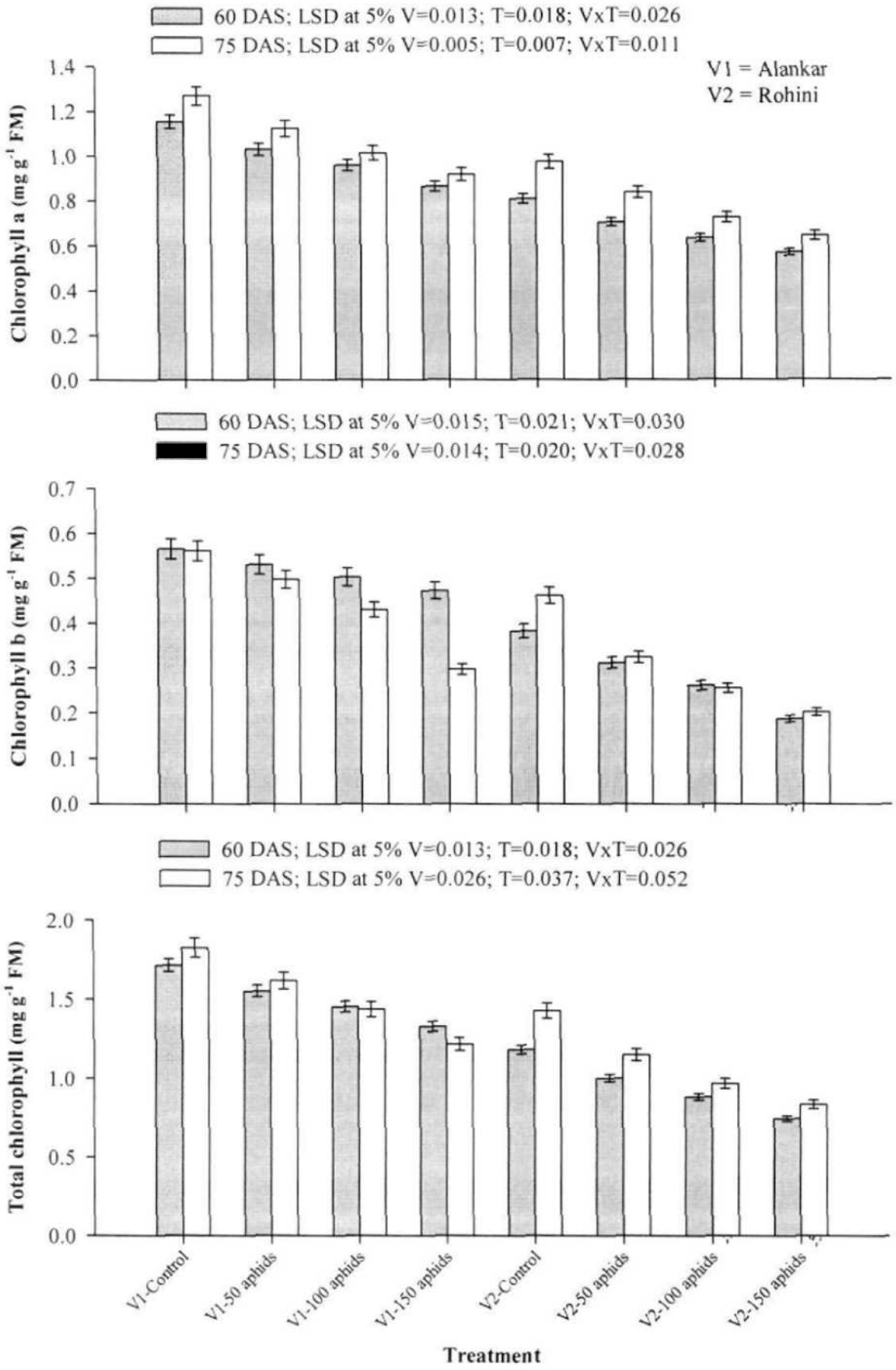


Fig. 21, 22, 23. Effect of aphid infestation (0, 50, 100 and 150 per plant) on chlorophyll a, b and total chlorophyll (mg g^{-1} FM) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

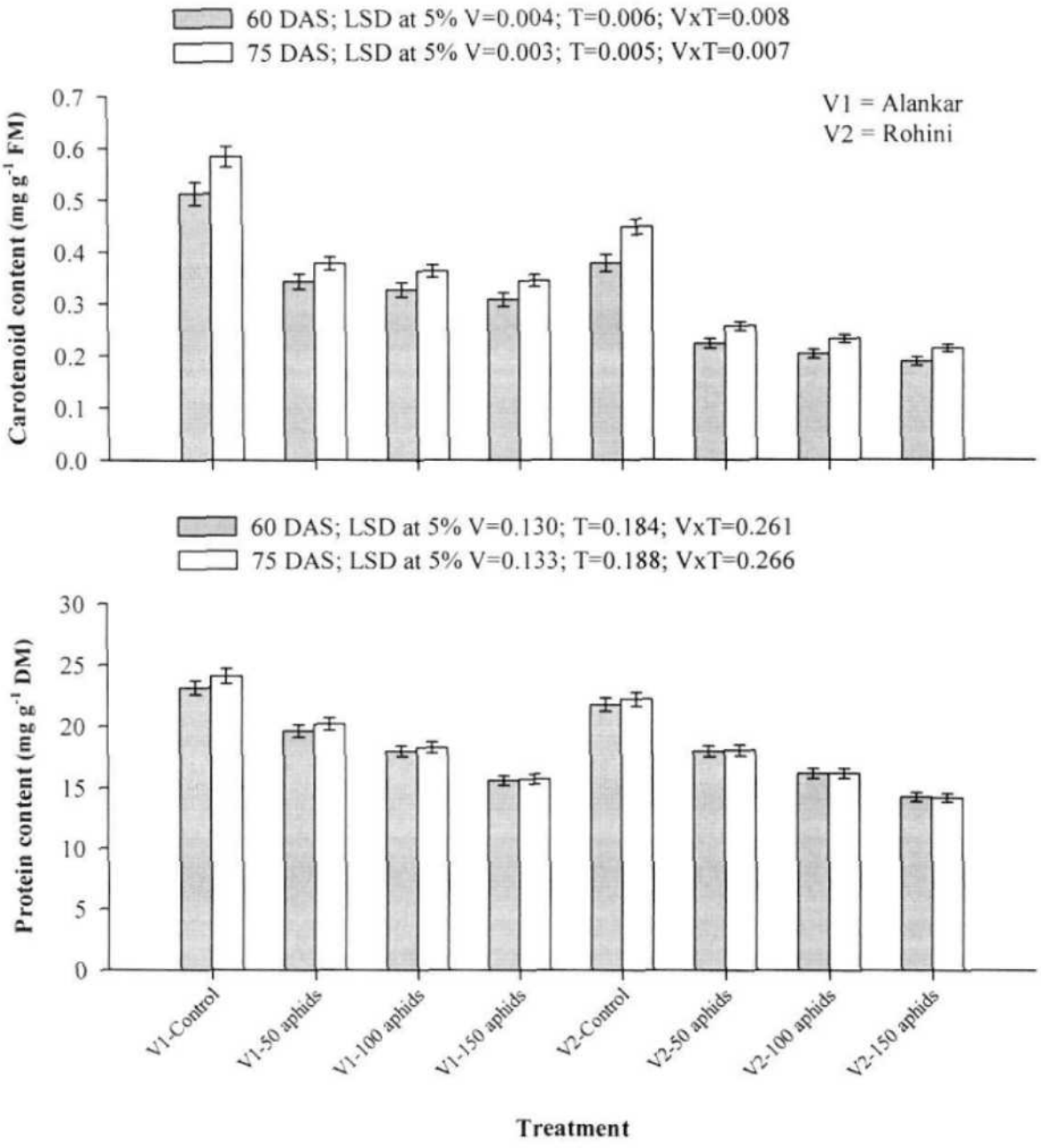


Fig. 24, 25 Effect of aphid infestation (0, 50, 100 and 150 per plant) on carotenoid content (mg g^{-1} FM) and protein content (mg g^{-1} DM) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

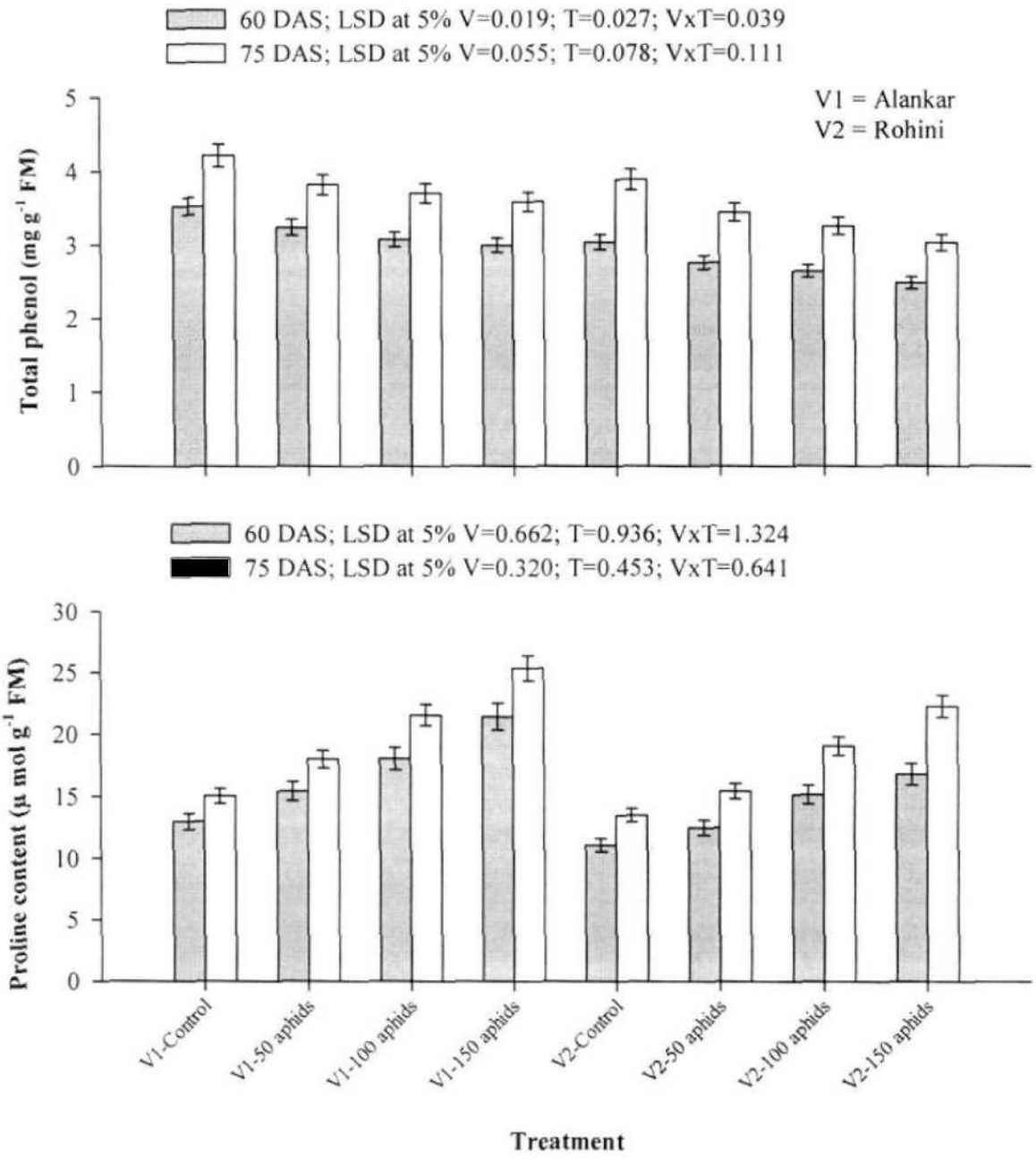


Fig. 26, 27. Effect of aphid infestation (0, 50, 100 and 150 per plant) on total phenol content (mg g^{-1} FM) and proline content (µ mol g^{-1} FM) and) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

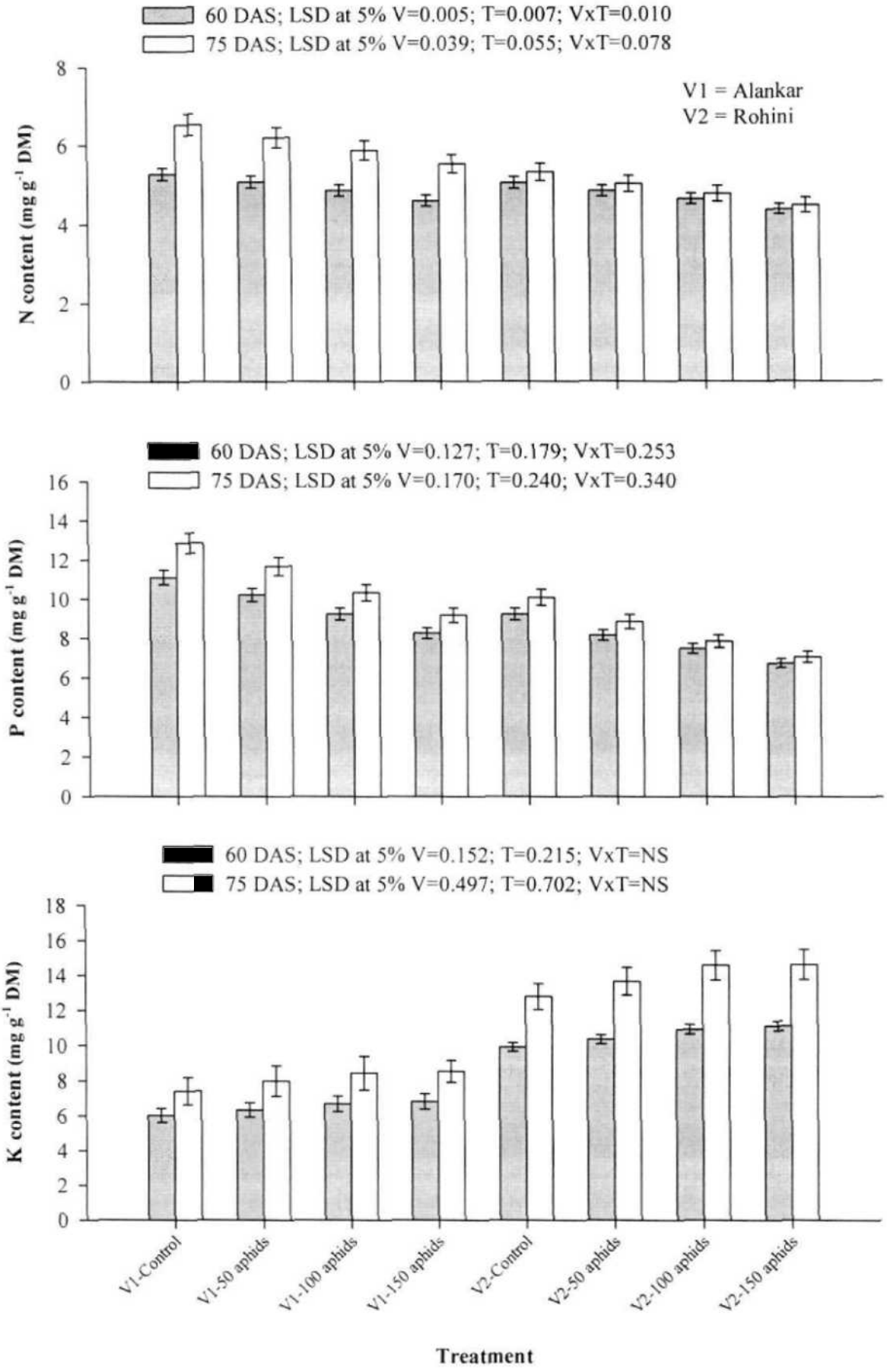


Fig. 28, 29, 30. Effect of aphid infestation (0, 50, 100 and 150 per plant) on N, P, and K content (mg g^{-1} DM) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

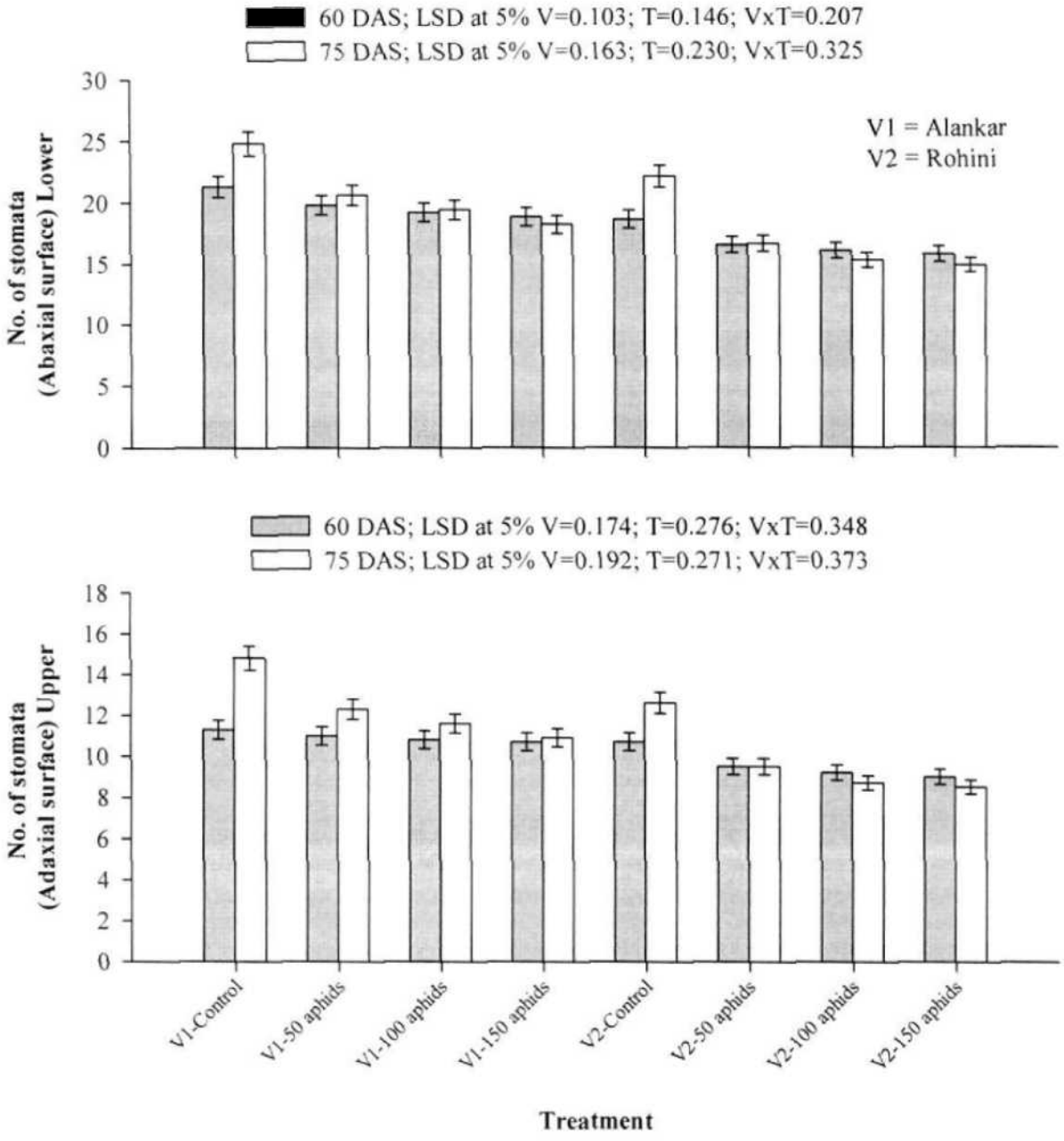


Fig. 31, 32. Effect of aphid infestation (0, 50, 100 and 150 per plant) on number of stomata on abaxial and adaxial leaf surface of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

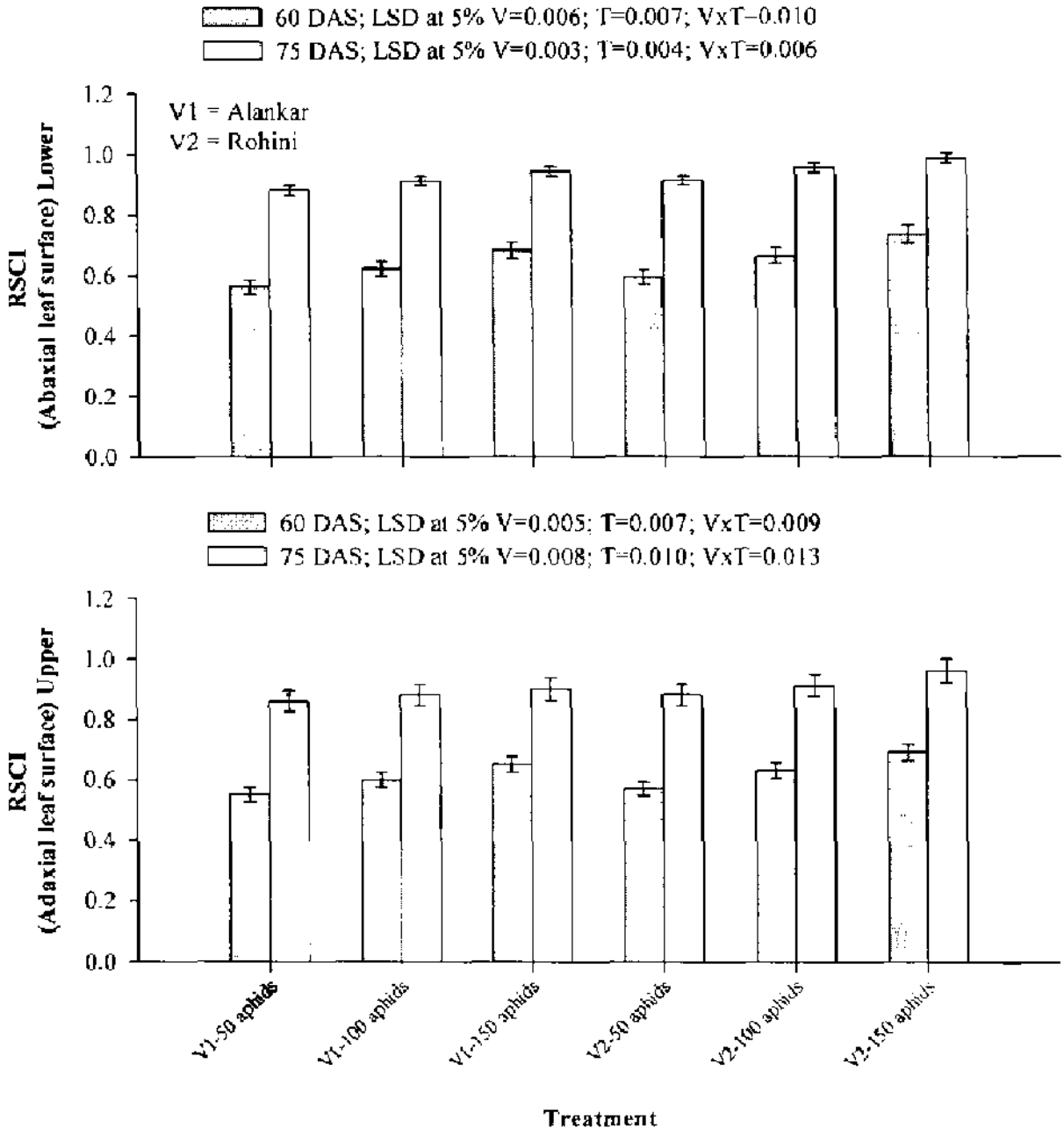


Fig. 33, 34. Effect of aphid infestation (0, 50, 100 and 150 per plant) on relative stomatal closure index (RSCI) of abaxial and adaxial surface of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

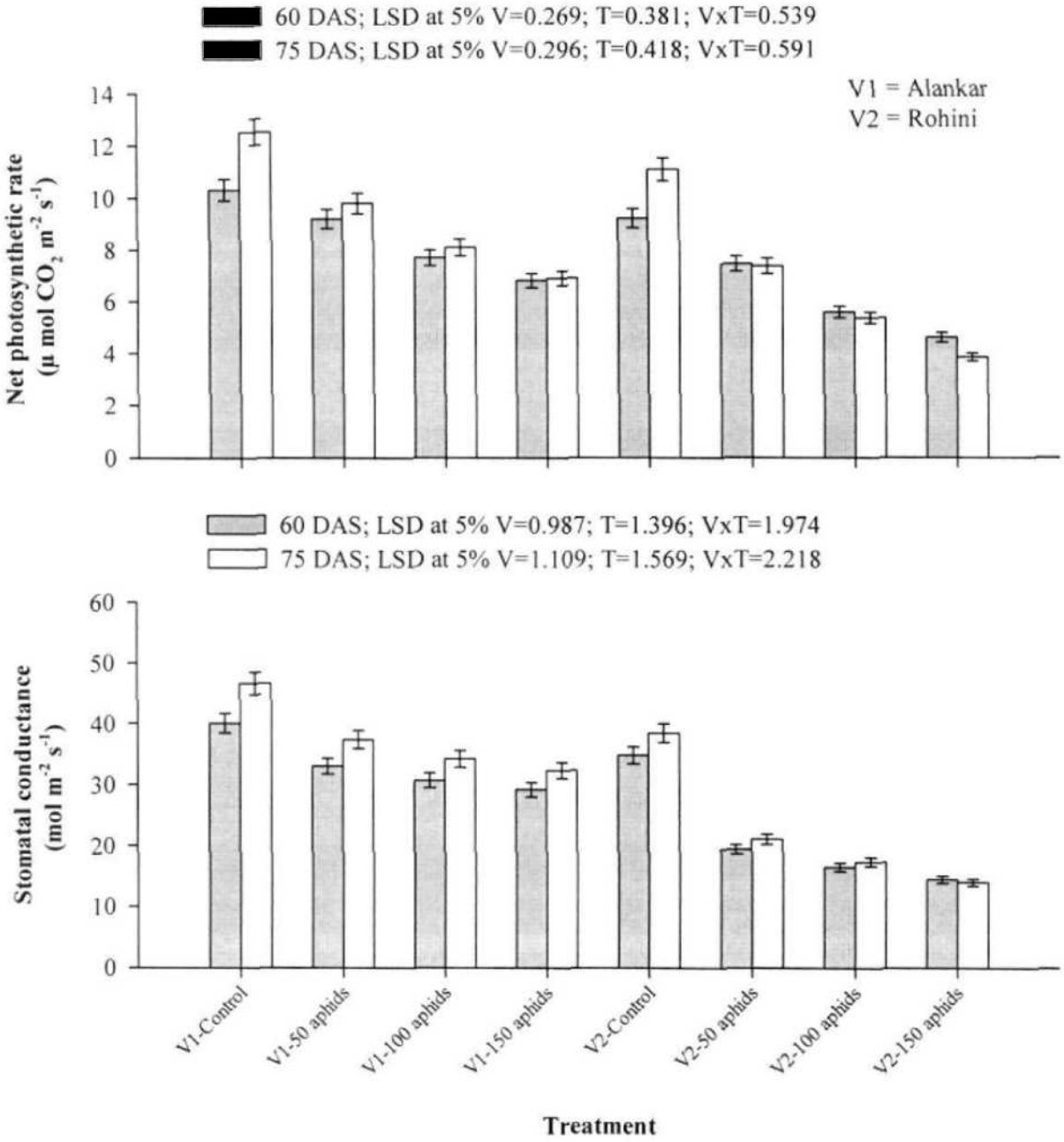


Fig. 35, 36. Effect of aphid infestation (0, 50, 100 and 150 per plant) on net photosynthetic rate (P_N; μ mol CO_2 m^{-2} sec^{-1}) and stomatal conductance (g_s; mol m^{-2} sec^{-1}) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

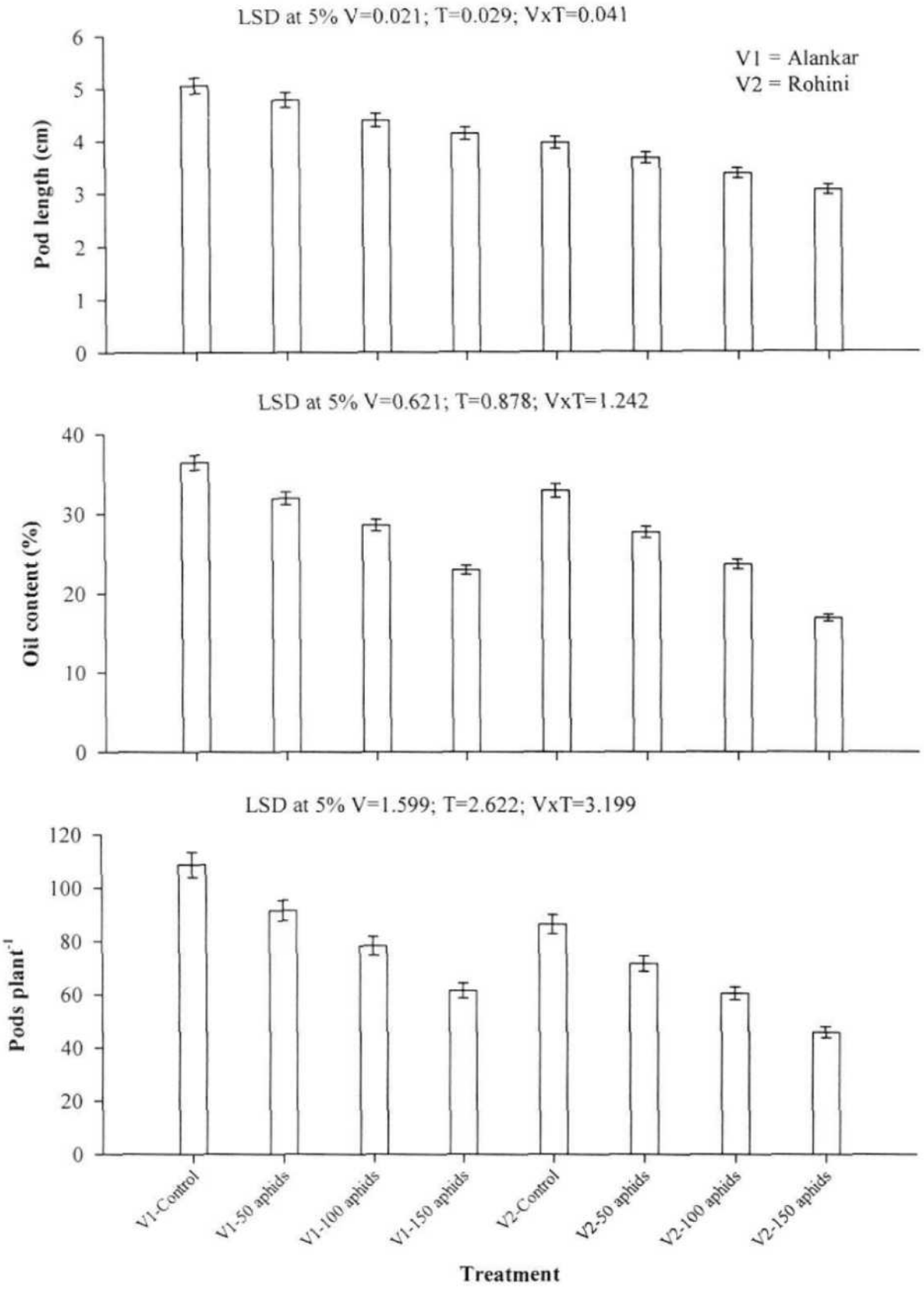


Fig. 37, 38, 39 Effect of aphid infestation (0, 50, 100 and 150 per plant) on pod length (cm), oil content (%) and pod plant^{-1} of *Brassica juncea* cvs. Alankar and Rohini at harvest (120 DAS)

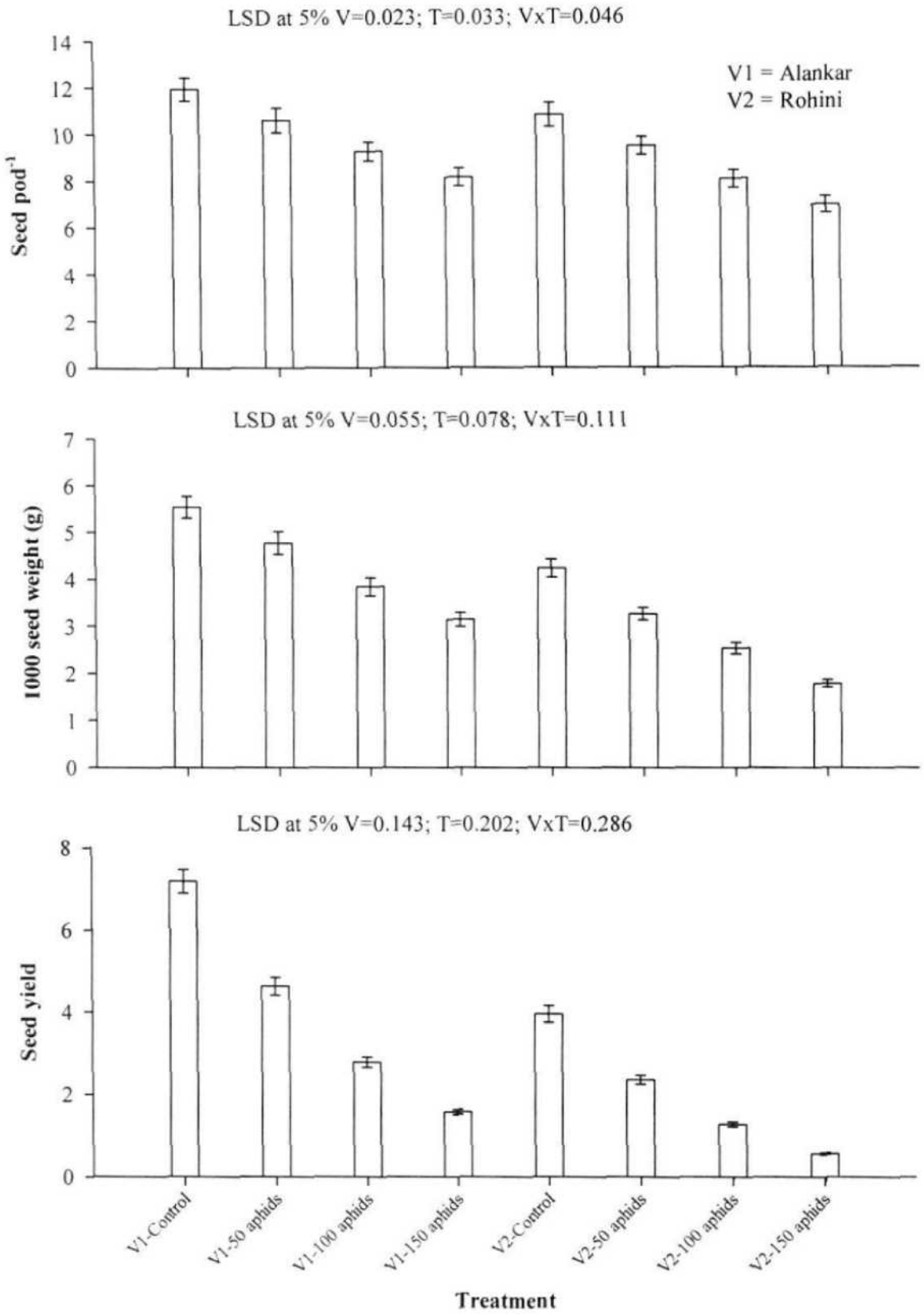


Fig. 40, 41, 42. Effect of aphid infestation (0, 50, 100 and 150 per plant) on yield characteristics of *Brassica juncea* cvs. Alankar and Rohini at harvest (120 DAS)

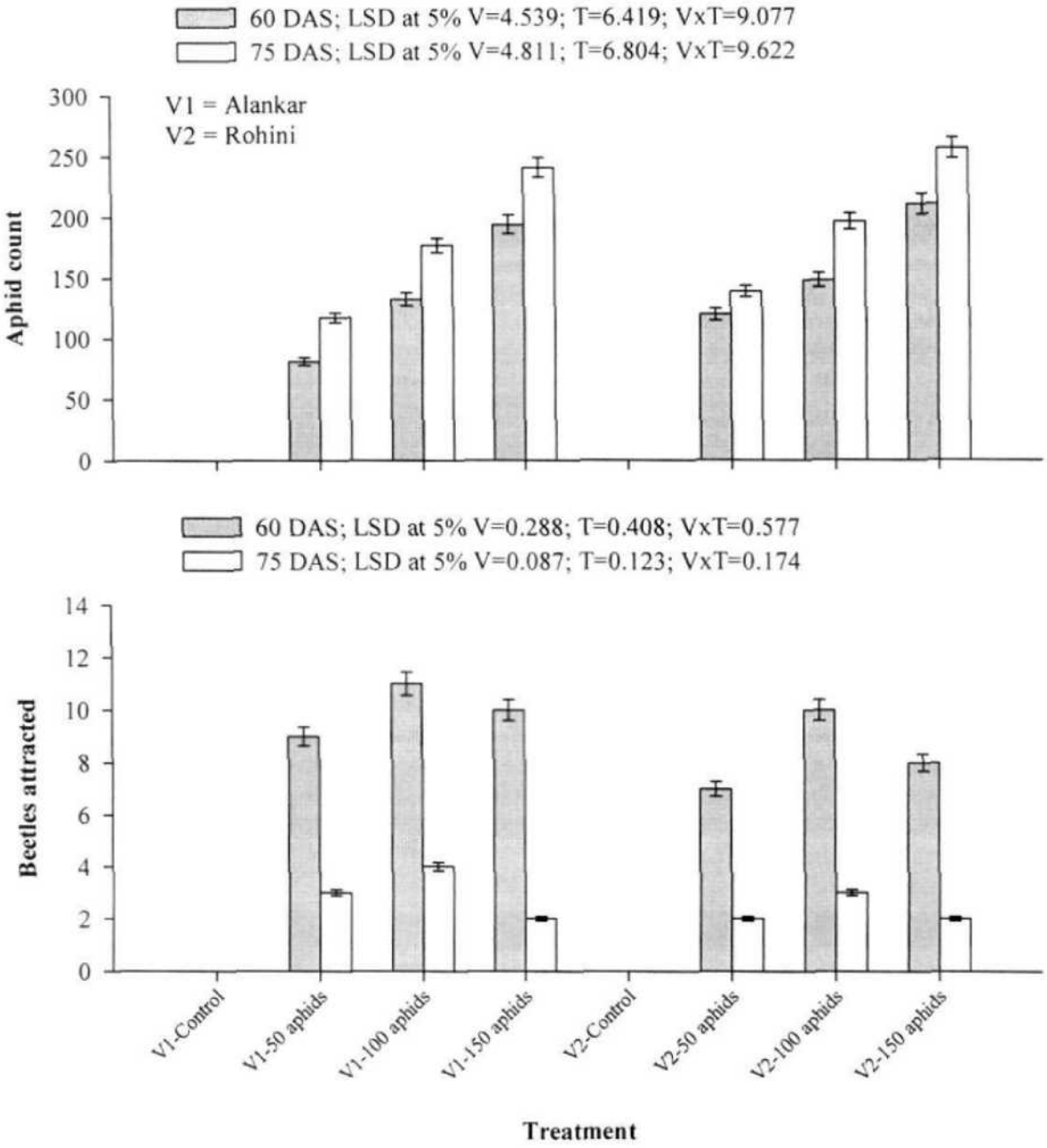


Fig. 43, 44 . Population count of aphid and number of beetle attracted after infestation on (0, 50, 100 and 150 per plant) on *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

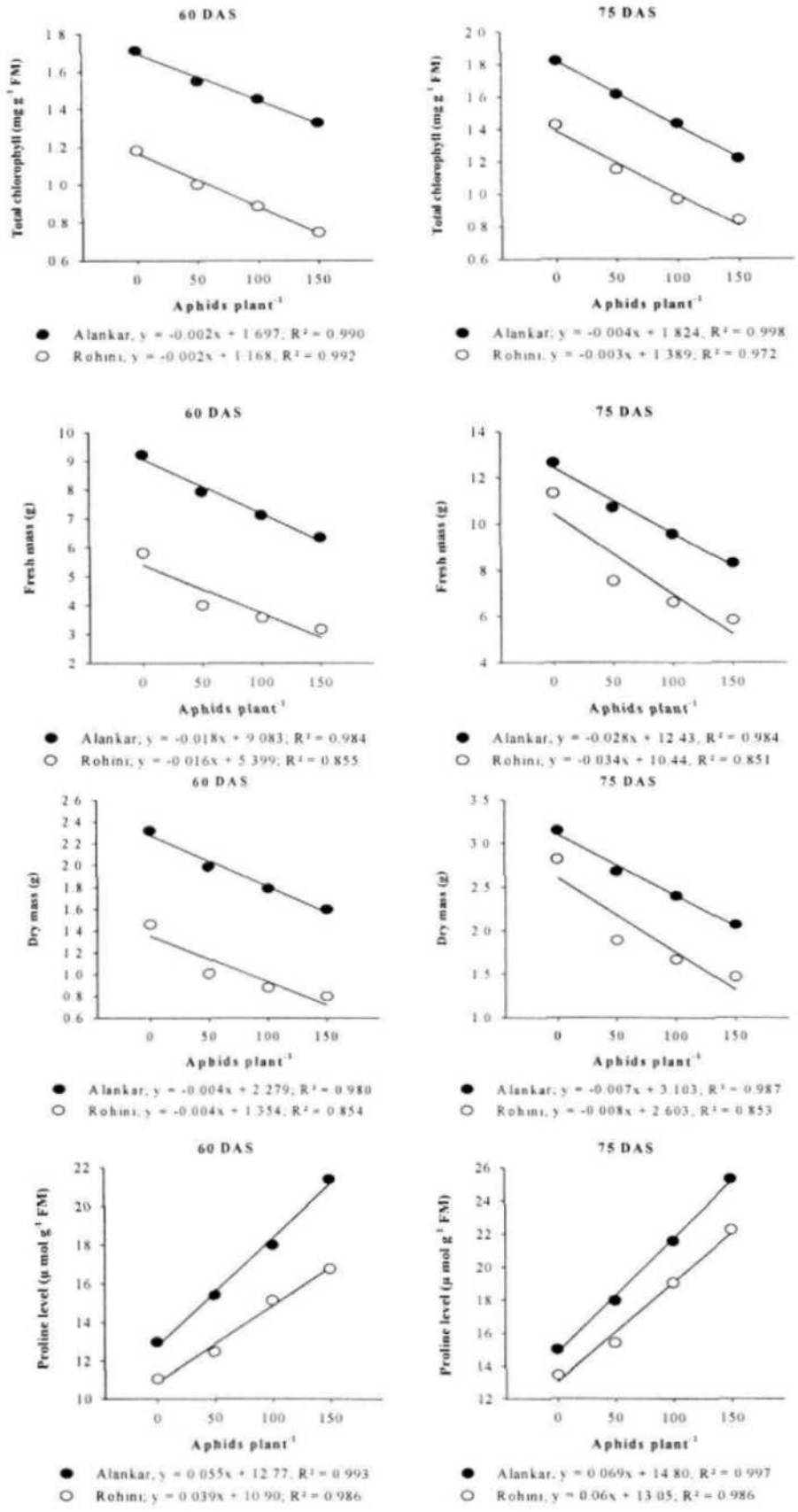


Fig.LR-III. Regression line showing the correlation coefficient between plant growth and biochemical parameters vs aphid population. viz., (A) total chlorophyll, (B) plant fresh mass (C) plant dry mass (D) proline content at 60 and 75 DAS

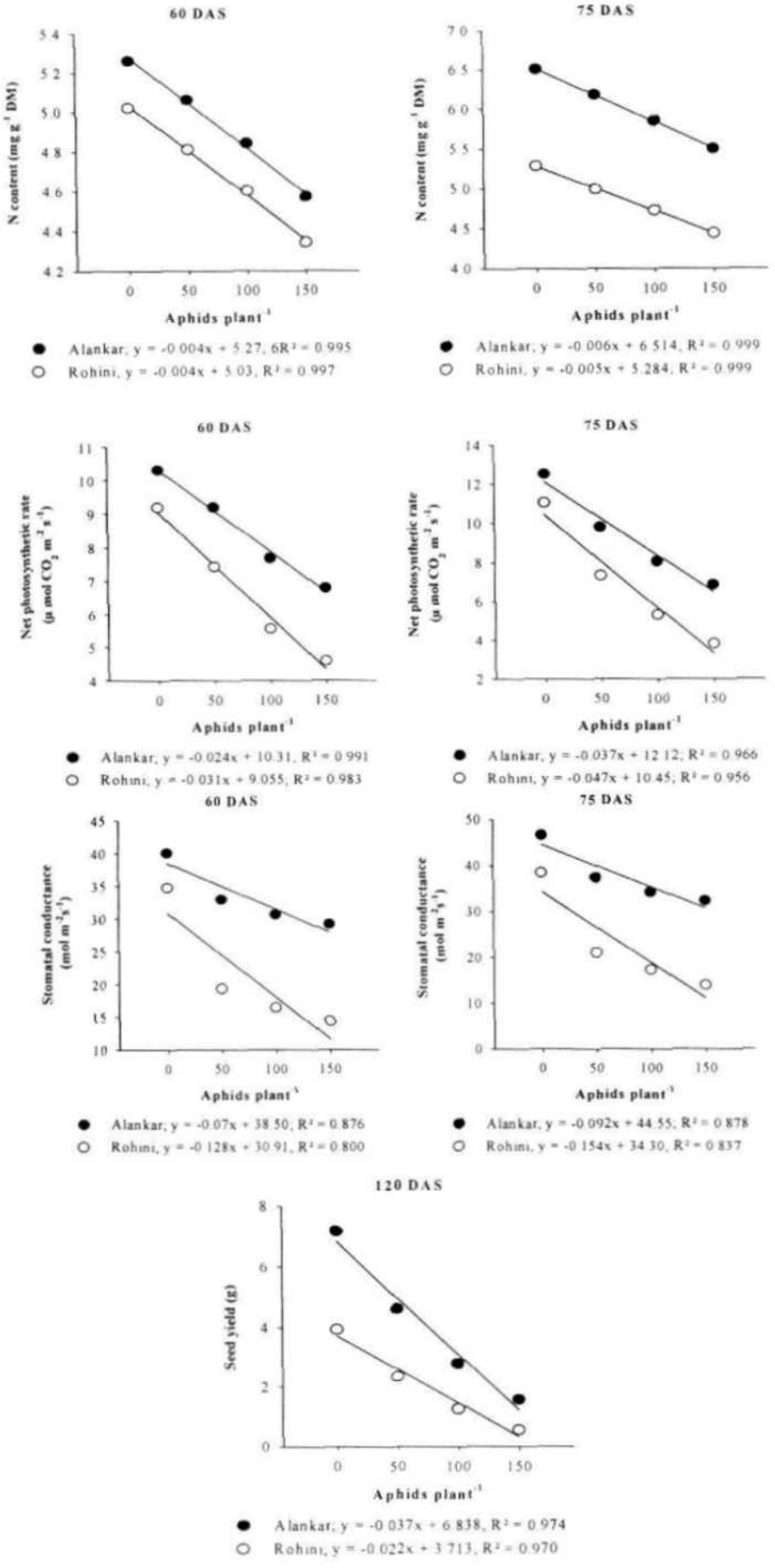


Fig.LR-IV. Regression line showing the correlation coefficient between plant growth and biochemical parameters vs aphid population. viz., (A) N content (B) net photosynthetic rate (C) stomatal conductance (D) seed yield at 60 and 75 DAS

attract beetles through their inherited defensive traits of signaling via volatile chemical signaling (VCS). The population of aphid and beetles attracted were counted at 60 and 75 DAS. The aphid injured cv. Alankar attracted larger number of aphids than by the cv. Rohini (Fig. 43, 44).

On the basis of per cent variation in all the parameters of cultivar Alankar and Rohini under varying levels of aphid herbivory, the responses of various parameters can be arranged in the following order of decreasing response. Leaf area > total chlorophyll > nitrogen > phosphorus > potassium.

Correlation coefficients and regression analysis

The correlation coefficient and regression line between selected parameters and aphid number per plant were determined. The total chlorophyll content, fresh mass, dry mass, nitrogen content and net photosynthesis rates, stomata conductance and seed yield had strong and negative correlation with number of aphid per plant at 60 and 75 DAS in both the cultivars. But the correlations between the growth variable of plant and aphid population were stronger in cv. Alankar than cv. Rohini. The proline content was positively correlated with the increase in aphid population and remaining parameters (total chlorophyll content, fresh mass, dry mass, nitrogen content and net photosynthesis rate, stomatal conductance and seed yield) had negative and significant correlation with aphid infestation level (Fig. LR III and IV).

Experiment -3

Plant responses to aphid herbivory along with predatory beetles

Both the selected cultivars were infested with varying levels of aphids (50, 100, 150 aphid per plant) and 5 days later equal level of predation was induced by introducing 2 beetles per plant. A set of control without aphids and beetles was also maintained. The shoot and root length consistently decreased with the increase in aphid infestation level despite equal level of predation by beetles. The reductions in shoot and root length were relatively higher in cv. Rohini than Alankar at 60 and 75 DAS (Fig. 45, 46). The impact of aphid infestation on shoot and root length were higher in cv. Rohini than cv. Alankar irrespective of predation of aphids by beetles and plant age. It is of significance to mention here that the per cent reductions in varying growth attributes as recorded in the present study were relatively lesser than

the reductions caused by equal number of aphid inoculation without beetles in the experiment 2.

The varying levels of aphid infestation adversely affected the leaf initiation and development as evident from the data on leaf number and leaf area per plant (Fig. 47, 48). The reductions in leaf number and leaf area corresponded with the levels of aphid infestation irrespective of predation by beetles. The impact of aphid infestation on the leaf area was relatively more severe at late stage (75 DAS) but, the leaf number did not show much variation at this stage (Fig. 47, 48). These data indicate that the expansion of leaf was more severely affected than the leaf initiation on aphid infestation and their predation by beetles (Fig. 47, 48).

The effects of varying levels of aphid herbivory on fresh and dry mass oh host plants are summarized in Fig. 49, 50. There were significant reductions in the fresh and dry mass of the plant. The reductions in fresh mass were higher in cv. Rohini than in Alankar at early stage but eventual loss in dry mass were relatively more severe at late stage (75 DAS) (Fig. 49, 50).

The impact of varying levels of aphid herbivory along with their predation by two beetles was studied on chlorophyll and carotenoid contents in two selected cultivars of mustard at two growth stages; the data are summarized in (Fig. 51-54). The aphid infestation affected chlorophyll a and b as well carotenoid content in proportion to their infestation level. The loss in chlorophyll increased with aphid number. The chlorophyll was more severely reduced in cv. Rohini than in Alankar. The predation of equal number of beetles did not apparently affected the severity of damage caused by aphids (Fig. 51-54).

In this experiment, the total protein and phenol content decreased in the plant (Fig. 55, 56). The protein was more severely affected in the most susceptible cultivar Rohini than Alankar even at early stage of growth (60 DAS) as evident from per cent loss with respect to control plant. The reductions in protein and phenol contents with respect to aphid infestation level were significant (Fig. 55, 56). The data on the accumulation of proline in the leaves of selected cultivars under varying levels of aphid infestation with predation by equal number of beetles are summarized in Fig. 57. The proline content increased in the plant with the level of aphid infestation stress and the predation by beetles. But the increase in proline content was more in cv.

Alankar (relatively resistant one) than in Rohini (susceptible). The accumulation of proline was higher at early stage of growth than the late stage (Fig. 57). Reductions in nitrogen, phosphorous and potassium (N, P and K) content in both the selected cultivars were also studied in the present experiment. The per cent reductions in NPK were relatively lesser than other parameters. The per cent reductions in nitrogen and phosphorous were marginal (Fig. 58-60).

The frequency and relative stomata closure index (RSCI), frequency of stomata on abaxial and adaxial surfaces of leaf were studied in the present experiment and the statistically analysed data are summarized in Fig. 61-64. The aphid infestation affected stomatal frequency more adversely in cv. Rohini than cv. Alankar. The reductions in stomatal frequency were higher at early stage (Fig. 61, 62). The stomatal closure was more severe at late stage and high level of aphid infestation. The relative stomatal closure index was almost equal on abaxial and adaxial surfaces of leaf but, differed significantly at each leaf surface on varying levels of aphid herbivory (Fig. 63, 64). The aphid infestation adversely affected the rate of net photosynthesis and stomata conductance. The reductions in rate of photosynthesis were in proportion to the level of aphid infestation (Fig. 65, 66). The stomatal conductance was more adversely impaired than the net rate of photosynthetic rate (Fig. 65, 66).

The data on pod length, oil content, pods per plant, seed per pod, mass of 1000 seeds and seed yield per plant were also collected and statistically analysed in the present study and are summarized in Figs. 67-72. The aphid infestation under beetle predation reduced pod length, pod formation, seed setting, seed development, seed yield and oil content adversely. The impact of aphid infestation was more severe on seed formation and development as well as oil content than the pod length, pods per plant and seeds per plant (Fig. 67-72).

Aphid demography

In the present experiment, the population of aphid was worked out in both the cultivars at 60 and 75 DAS. The aphid population was considerably low in cv. Alankar than in cv. Rohini (Fig. 73) as compared to the aphid population count in experiment 2 conducted with aphid herbivory alone indicating that beetles reduced the aphid population.

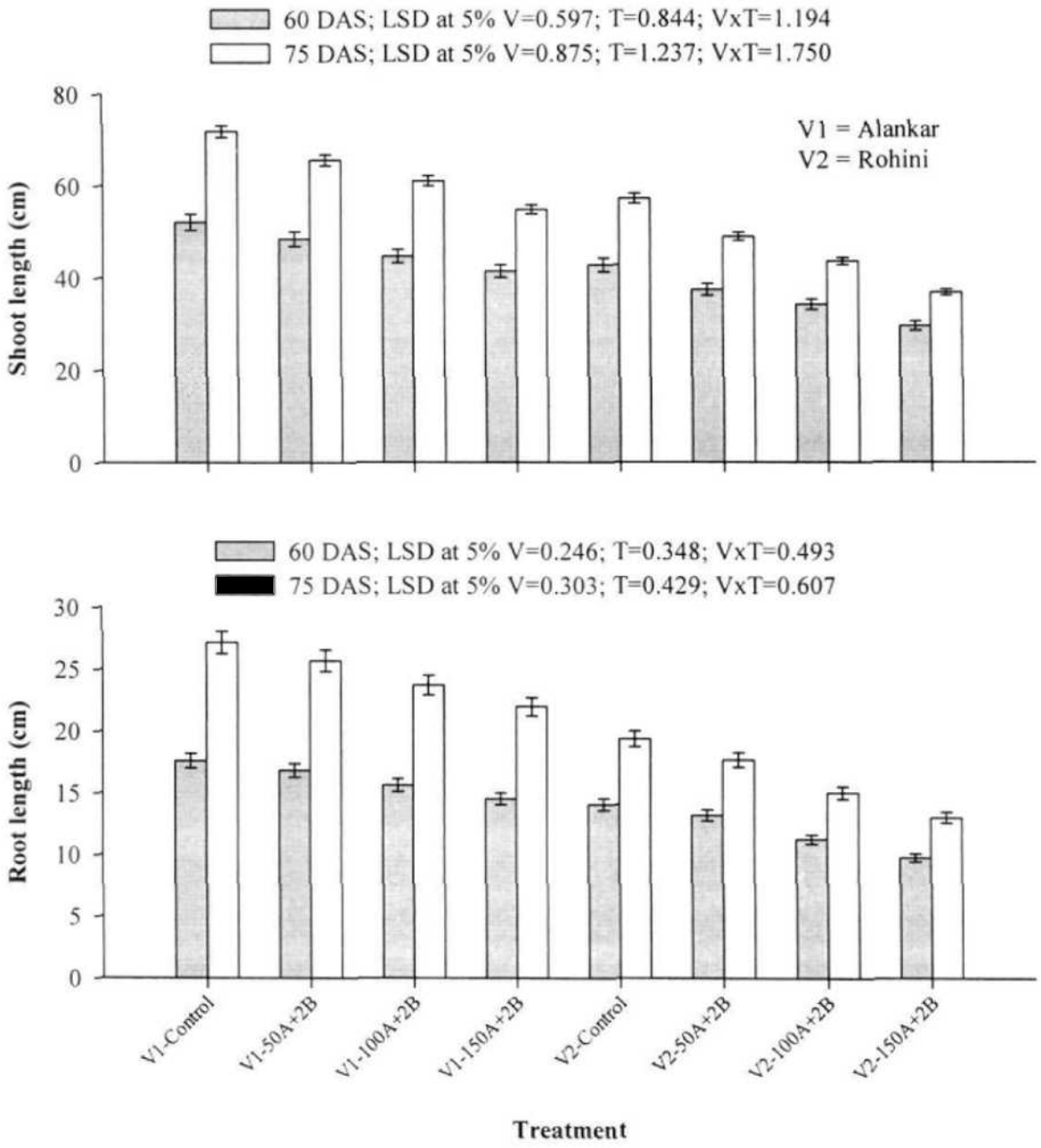


Fig. 45, 46. Responses of shoot and root length (cm) of *Brassica juncea* cvs. Alankar and Rohini exposed at 45 DAS to varying level of aphid infestations (0, 50, 100, 150 aphid per plant) and successive predation of aphids (5 days after) by 2 beetle per plant studied at 60 and 75 DAS.

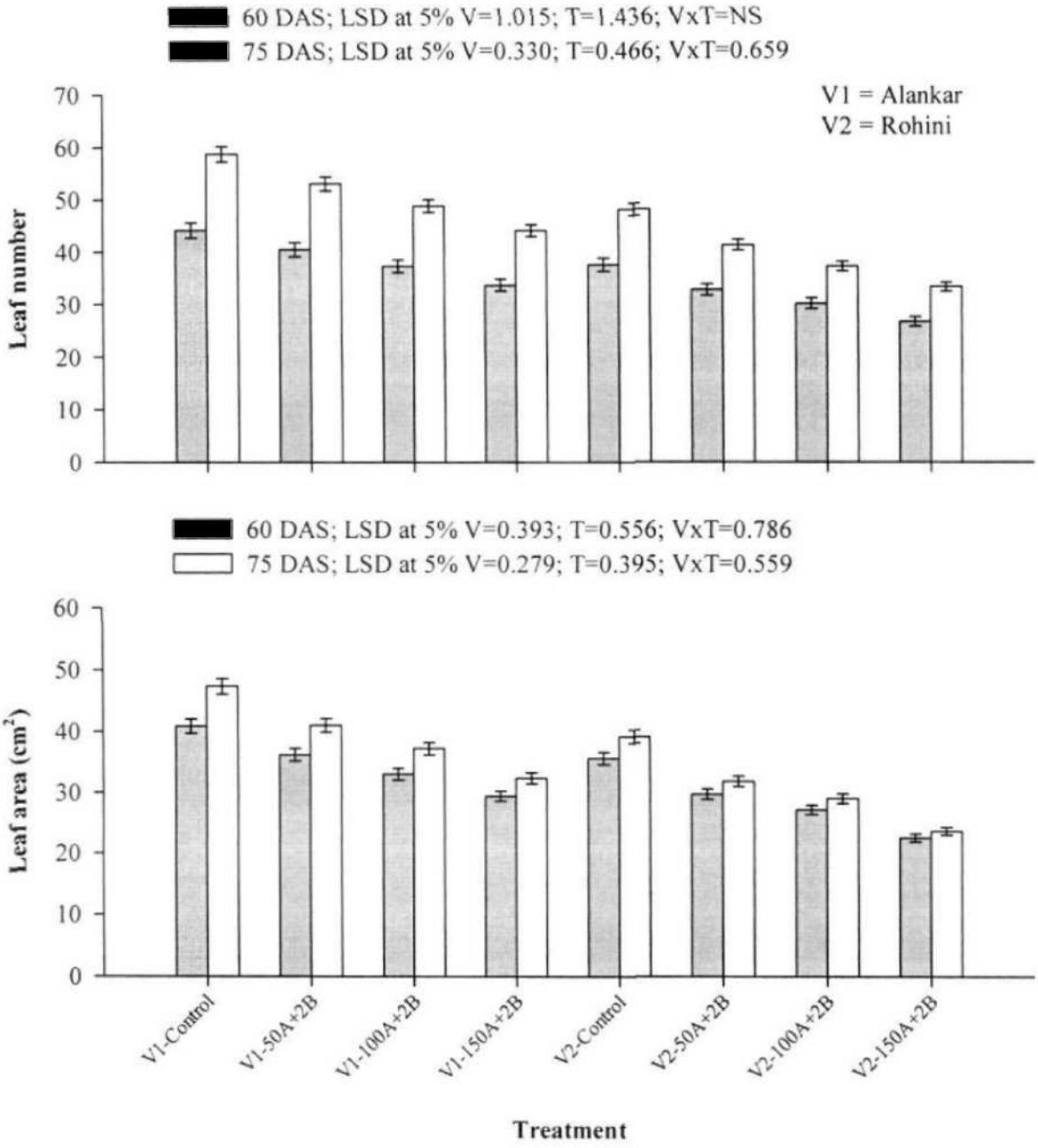


Fig. 47, 48. Responses of leaf number and area (cm^2) of *Brassica juncea* cvs. Alankar and Rohini exposed at 45 DAS to varying level of aphid infestations (0, 50, 100, 150 aphid per plant) and successive predation of aphids (5 days after) by 2 beetle per plant studied at 60 and 75 DAS.

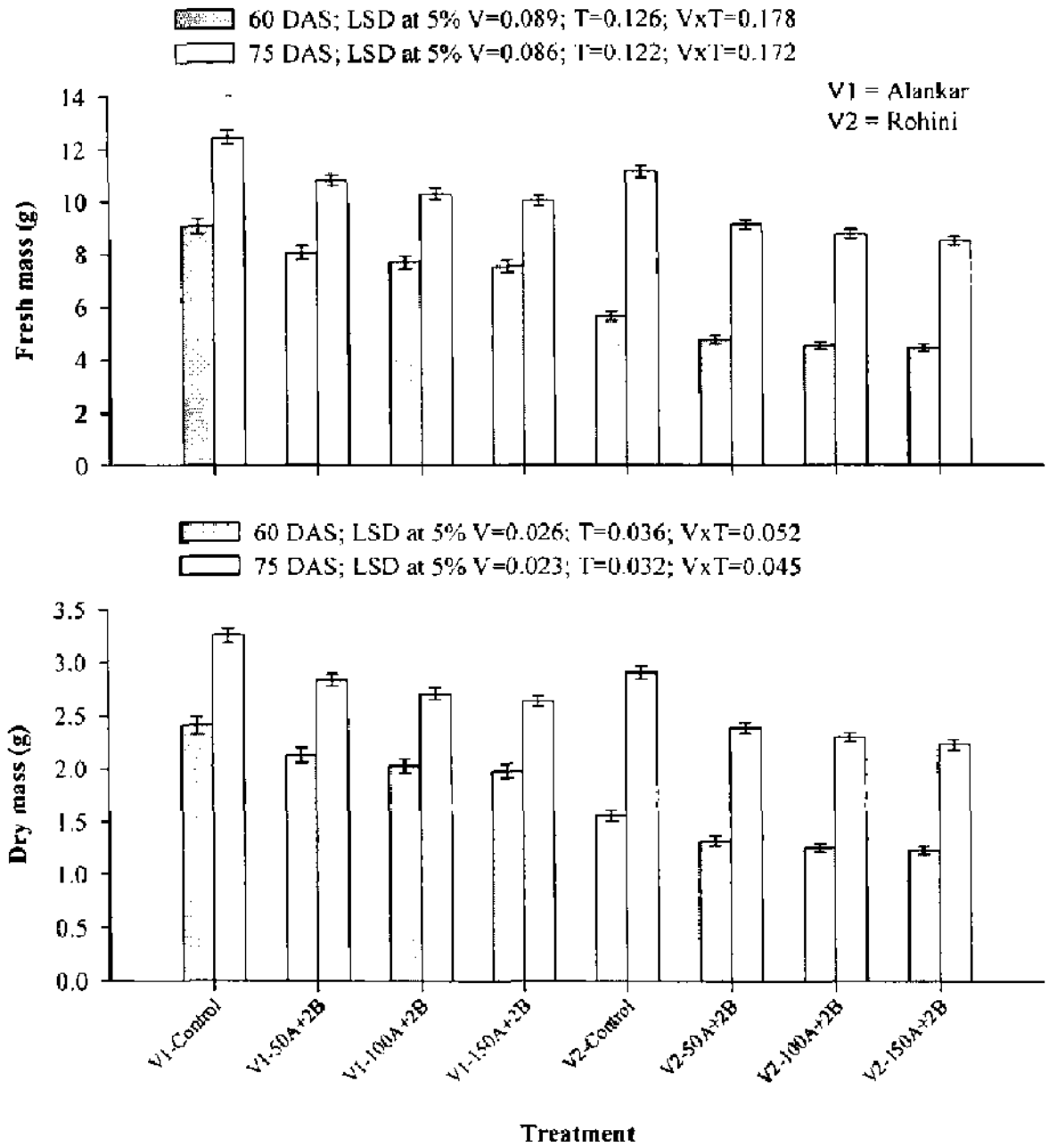


Fig. 49, 50. Responses of fresh and dry mass (g) of *Brassica juncea* cvs. Alankar and Rohini exposed at 45 DAS to varying level of aphid infestations (0, 50, 100, 150 aphid per plant) and successive predation of aphids (5 days after) by 2 beetle per plant studied at 60 and 75 DAS.

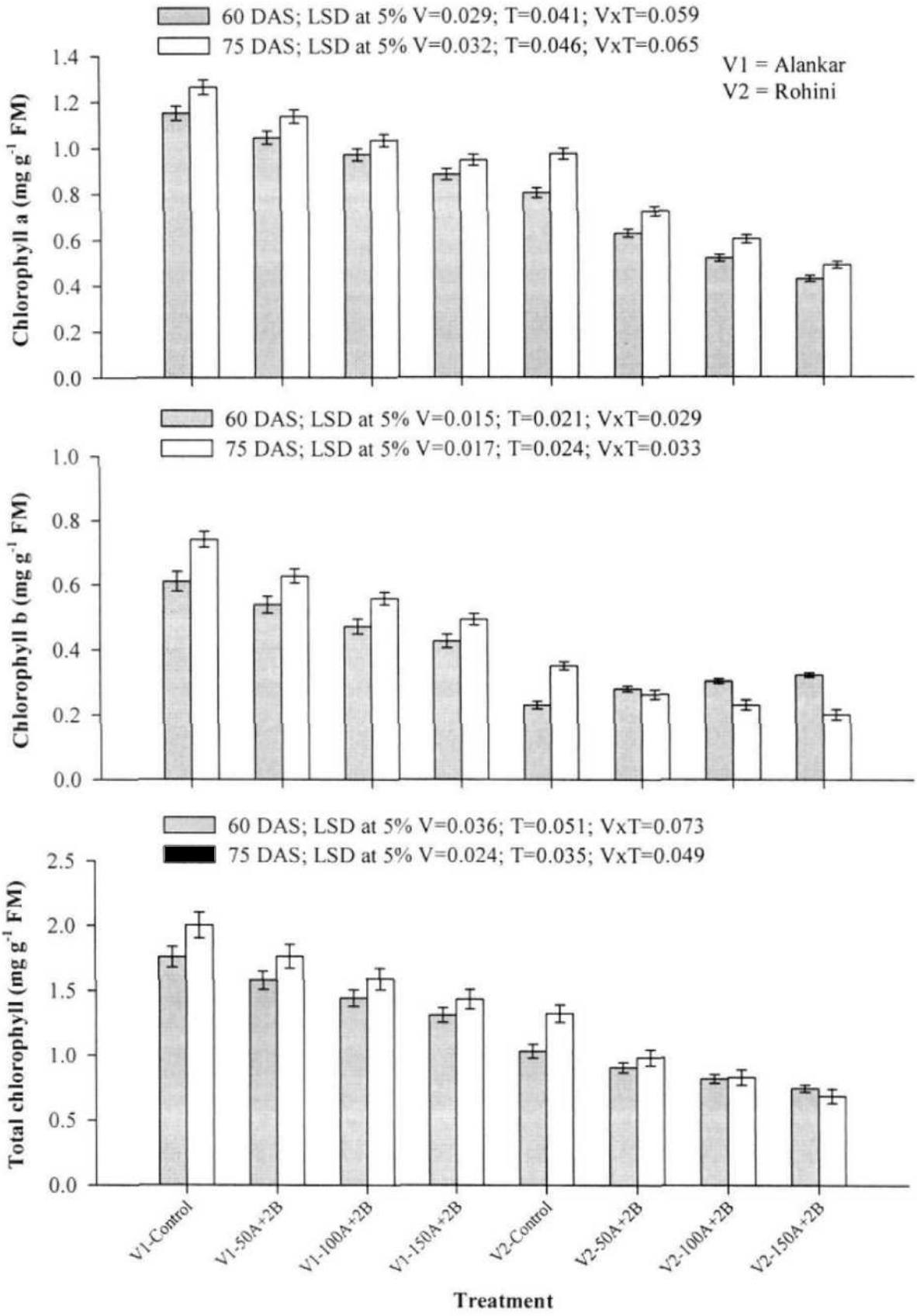


Fig. 51, 52, 53. Responses of chlorophyll a, b and total chlorophyll content (mg g^{-1} FM) of *Brassica juncea* cvs. Alankar and Rohini exposed at 45 DAS to varying level of aphid infestations (0, 50, 100, 150 aphid per plant) and successive predation of aphids (5 days after) by 2 beetle per plant studied at 60 and 75 DAS

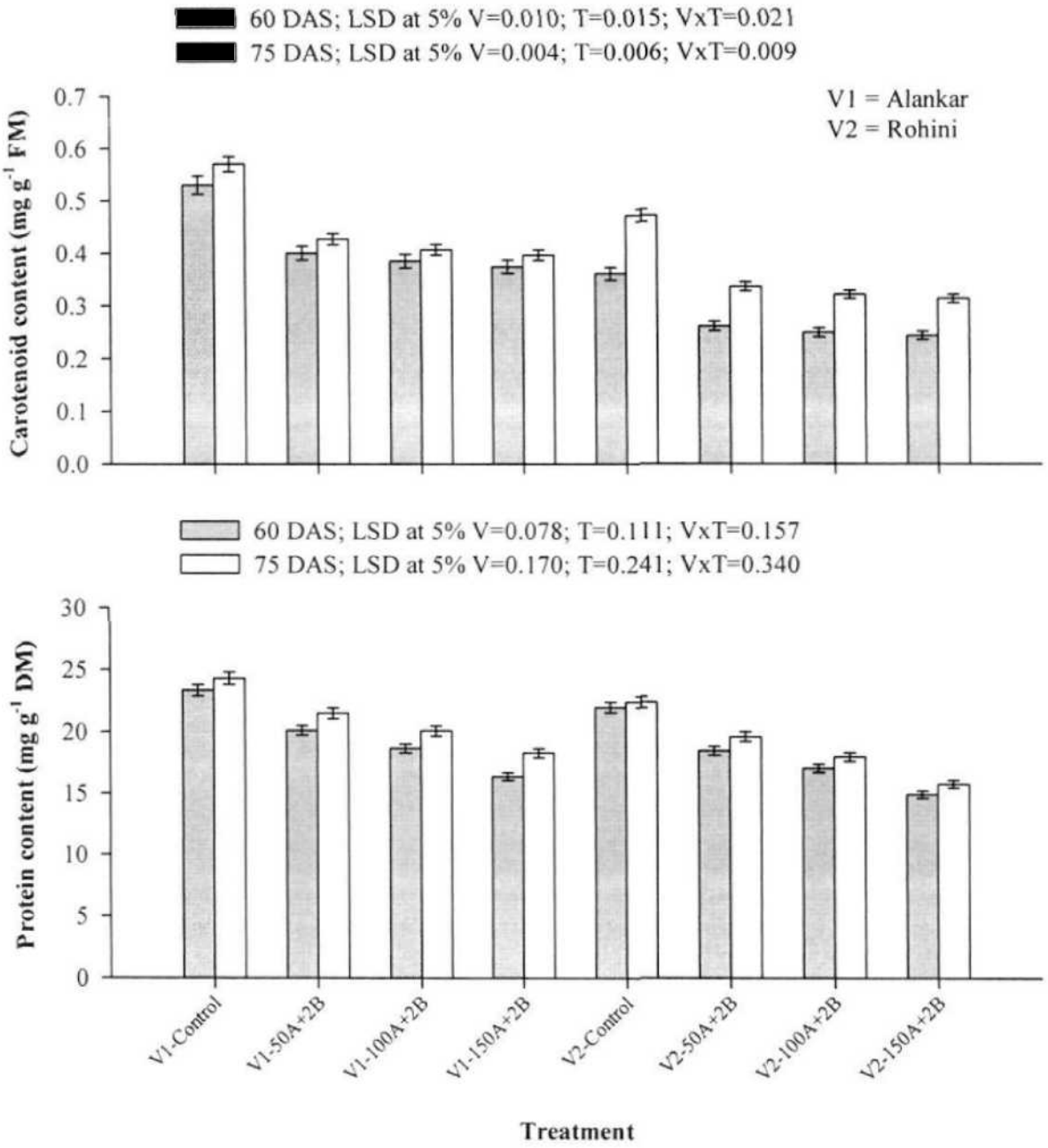


Fig. 54, 55. Responses of carotenoid content (mg g^{-1} FM) and protein content (mg g^{-1} DM) of *Brassica juncea* cvs. Alankar and Rohini exposed at 45 DAS to varying level of aphid infestations (0, 50, 100, 150 aphid per plant) and successive predation of aphids (5 days after) by 2 beetle per plant studied at 60 and 75 DAS.

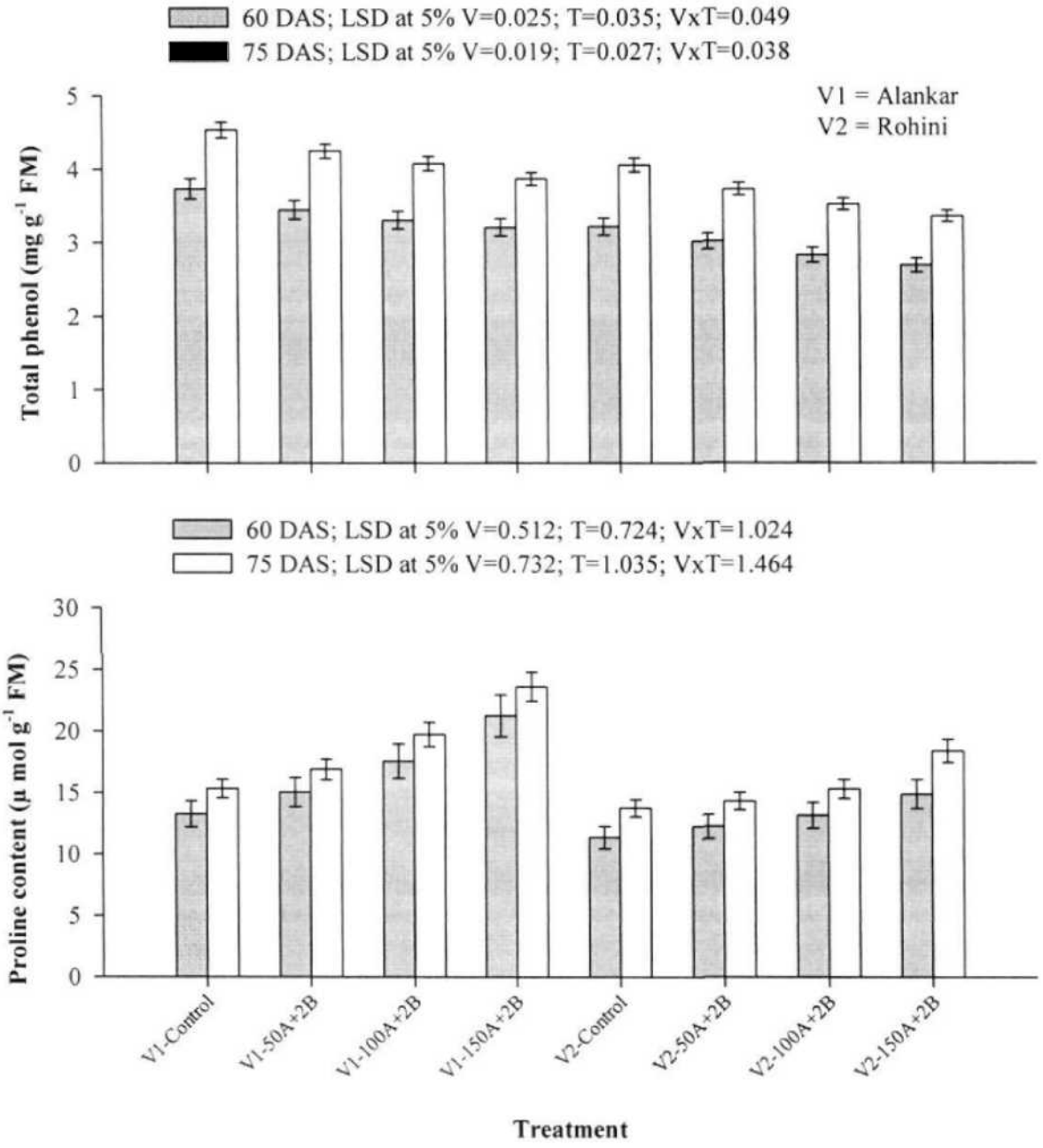


Fig. 56, 57. Responses of total phenol content and proline content (µ mol g^{-1} FM) of *Brassica juncea* cvs. Alankar and Rohini exposed at 45 DAS to varying level of aphid infestations (0, 50, 100, 150 aphid per plant) and successive predation of aphids (5 days after) by 2 beetle per plant studied at 60 and 75 DAS.

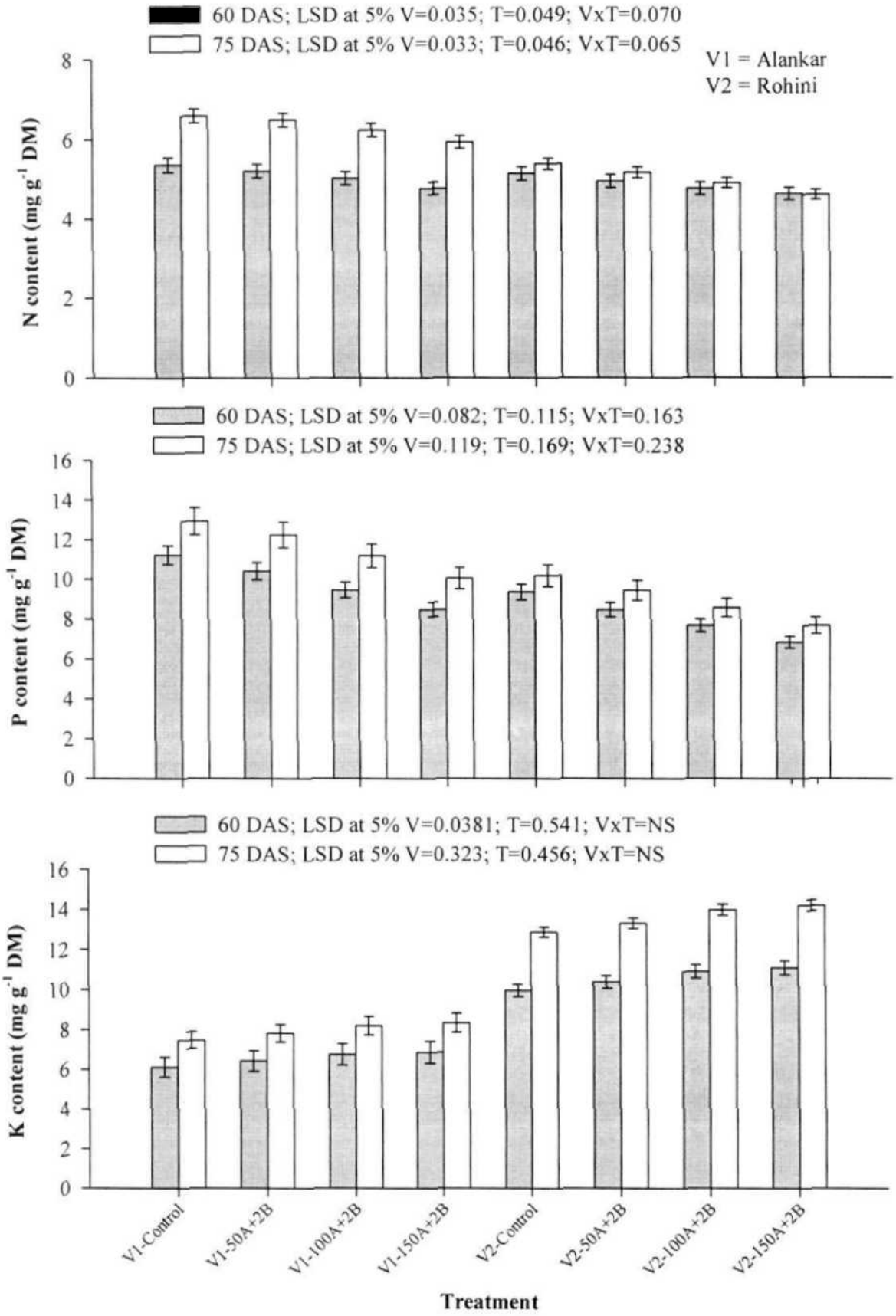


Fig. 58, 59, 60 Responses of N, P, and K content (mg g^{-1} DM) of *Brassica juncea* cvs. Alankar and Rohini exposed at 45 DAS to varying level of aphid infestations (0, 50, 100, 150 aphid per plant) and successive predation of aphids (5 days after) by 2 beetle per plant studied at 60 and 75 DAS.

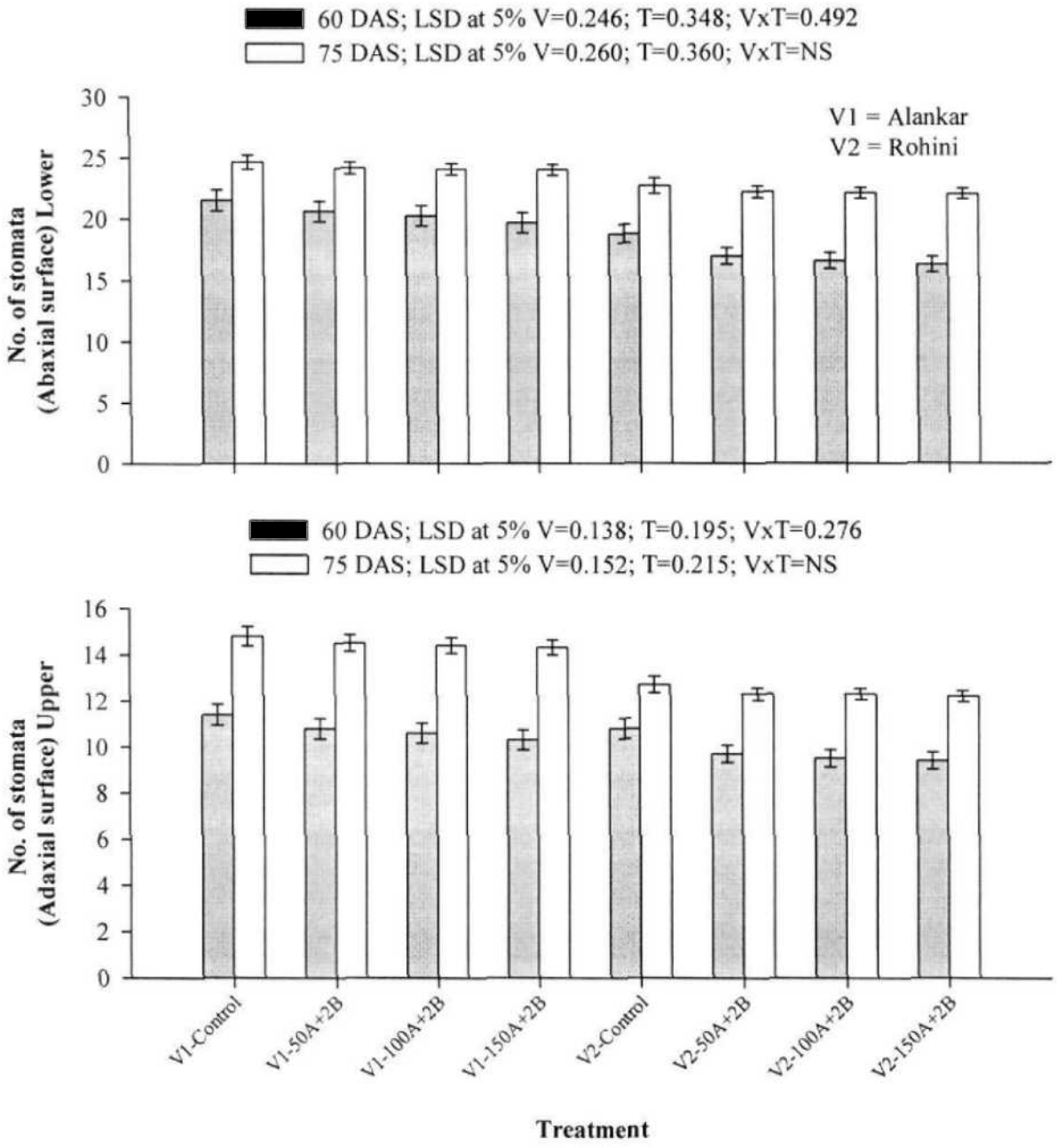


Fig. 61, 62. Responses of number of stomata on abaxial and adaxial surface of *Brassica juncea* cvs. Alankar and Rohini exposed at 45 DAS to varying level of aphid infestations (0, 50, 100, 150 aphid per plant) and successive predation of aphids (5 days after) by 2 beetle per plant studied at 60 and 75 DAS.

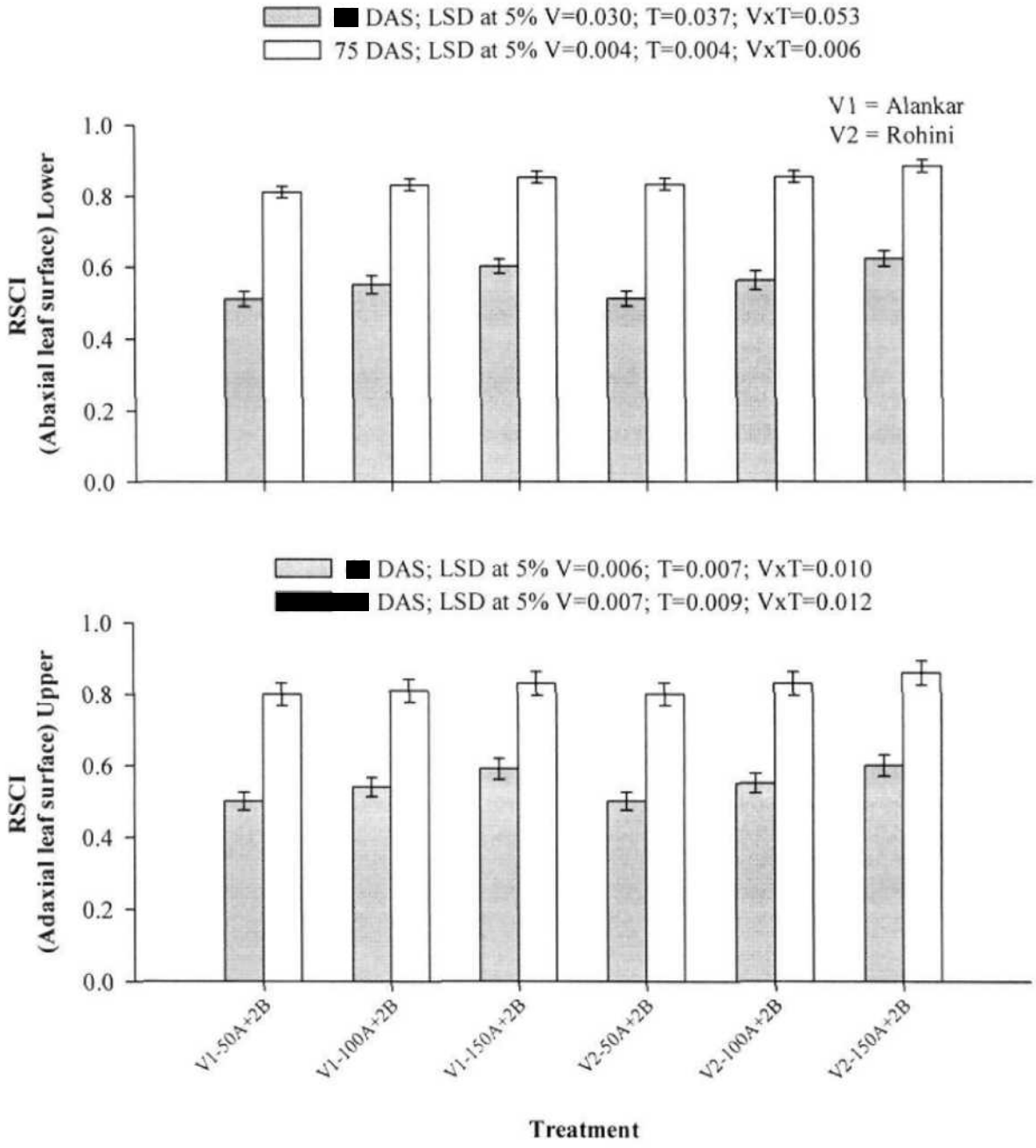


Fig. 63, 64. Responses of relative stomatal closure index (RSCI) of abaxial and adaxial surface of Brassica *juncea* cvs. Alankar & Rohini exposed at 45 DAS to varying level of aphid infestations (0, 50, 100, 150 aphid per plant) and successive predation of aphids (5 days after) by 2 beetle per plant studied at 60 and 75 DAS

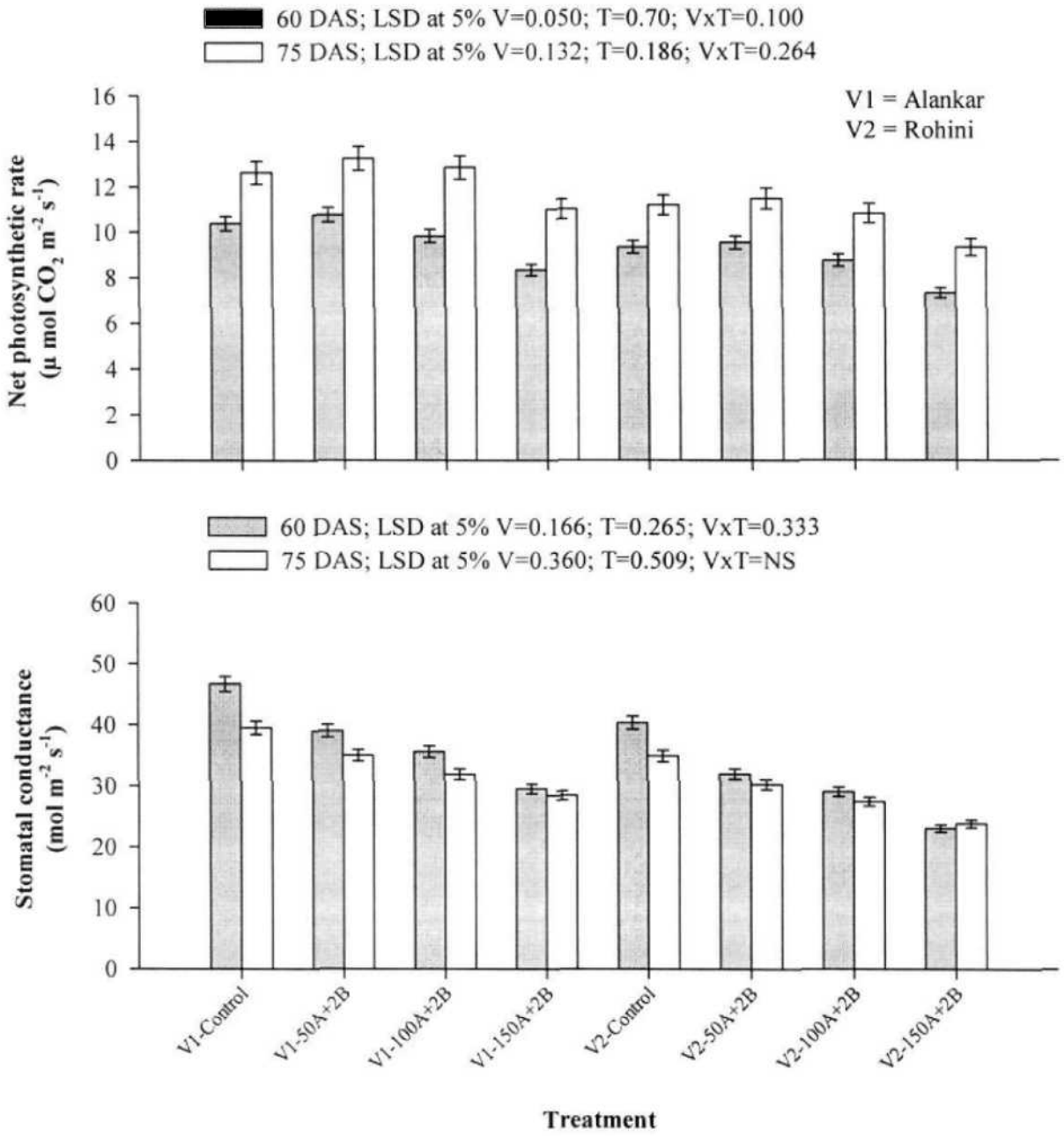


Fig. 65, 66 Responses of net photosynthetic rate (P_N; μ mol CO_2 m^{-2} sec^{-1}) and stomata conductance (g_s; mol m^{-2} sec^{-1}) *Brassica juncea* cvs. Alankar and Rohini exposed at 45 DAS to varying level of aphid infestations (0, 50, 100, 150 aphid per plant) and successive predation of aphids (5 days after) by 2 beetle per plant studied at 60 and 75 DAS

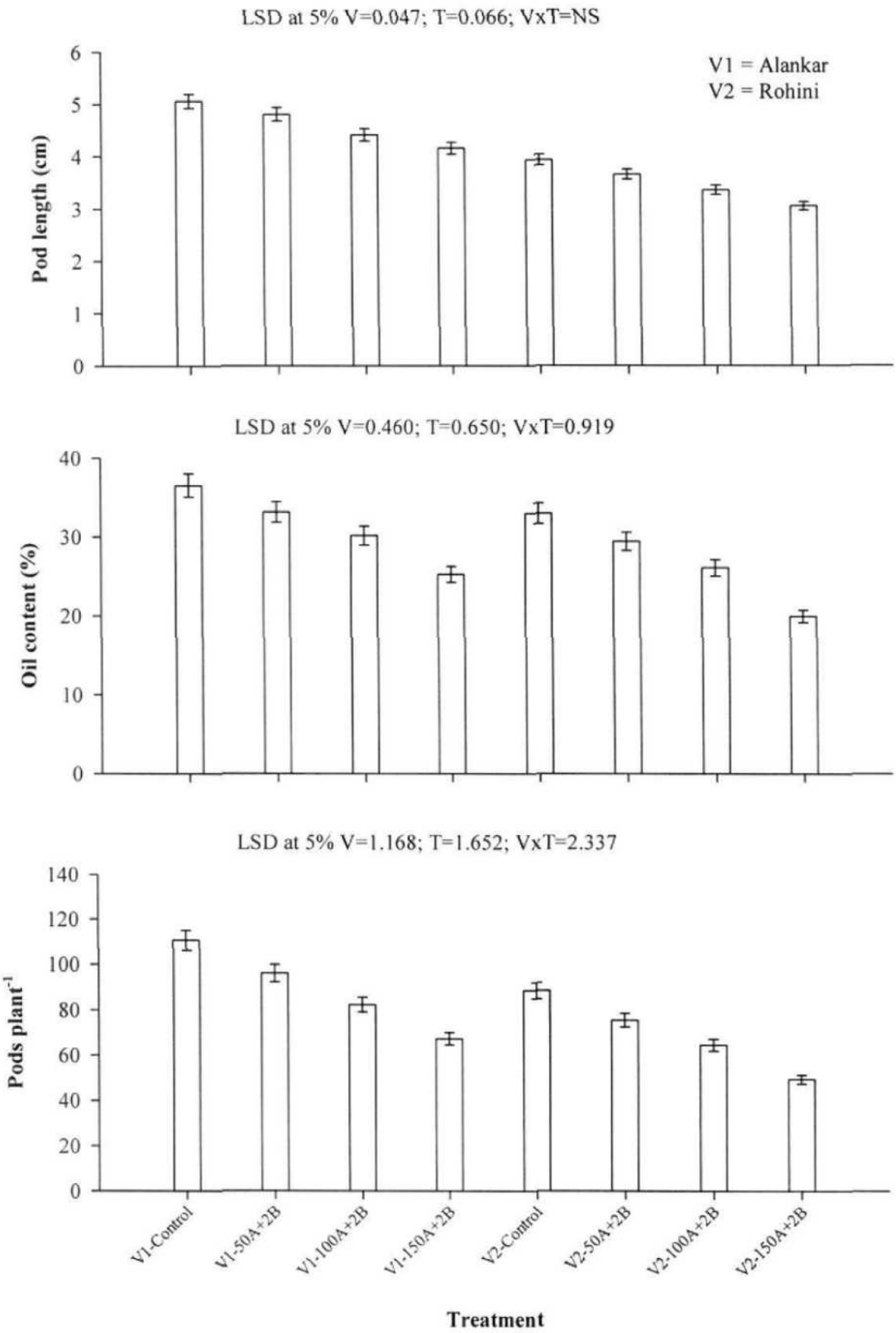


Fig. 67, 68, 69. Responses of pod length (cm), oil content (%) and pods plant^{-1} of *Brassica juncea* cvs. Alankar and Rohini exposed at 45 DAS to varying level of aphid infestations (0, 50, 100, 150 aphid per plant) and successive predation of aphids (5 days after) by 2 beetle per plant studied at 60 and 75 DAS.

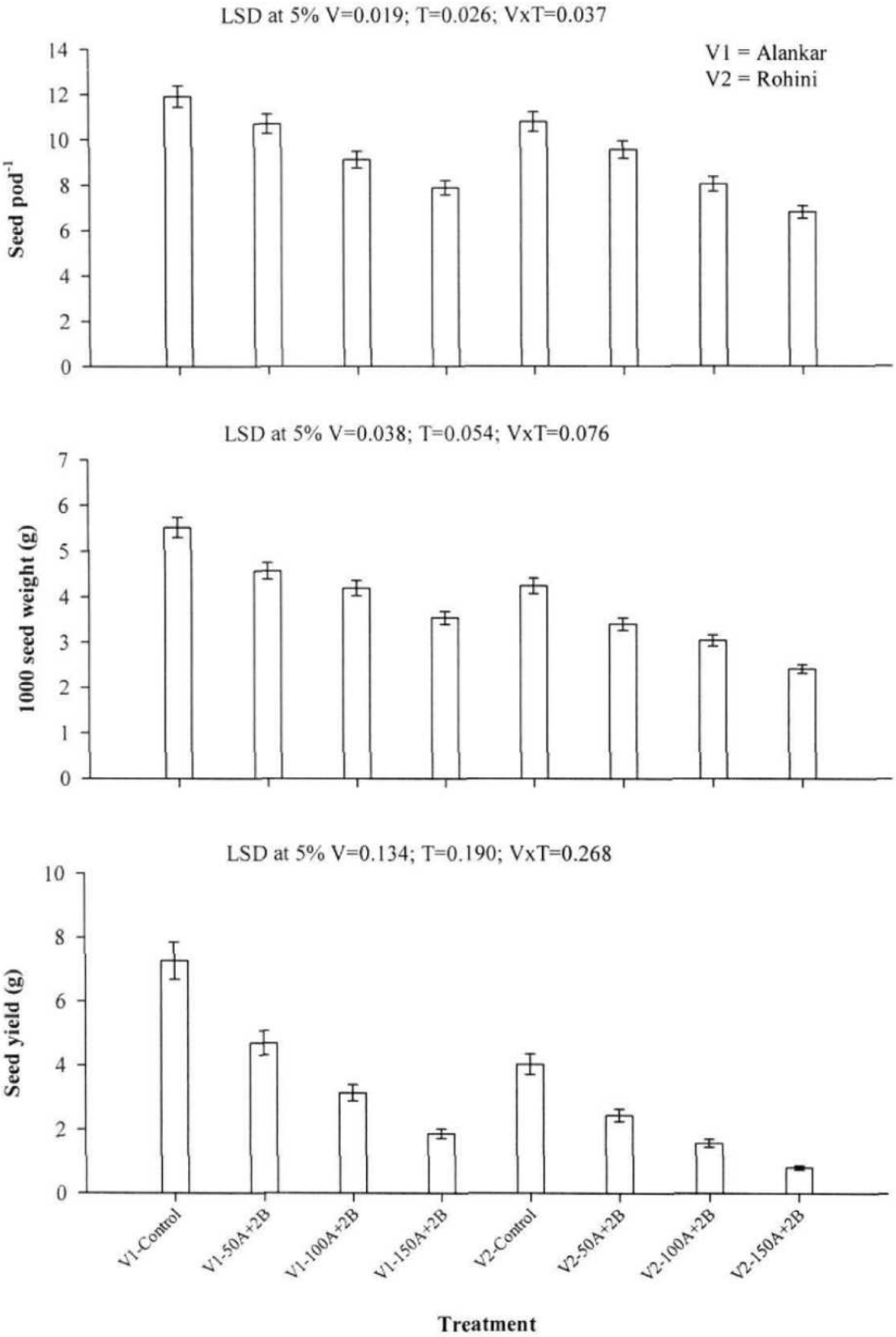


Fig. 70, 71, 72. Responses of seed pod^{-1}, 1000 seed weight (g) and seed yield (g) of *Brassica juncea* cvs. Alankar and Rohini exposed at 45 DAS to varying level of aphid infestations (0, 50, 100, 150 aphid per plant) and successive predation of aphids (5 days after) by 2 beetle per plant studied at 60 and 75 DAS

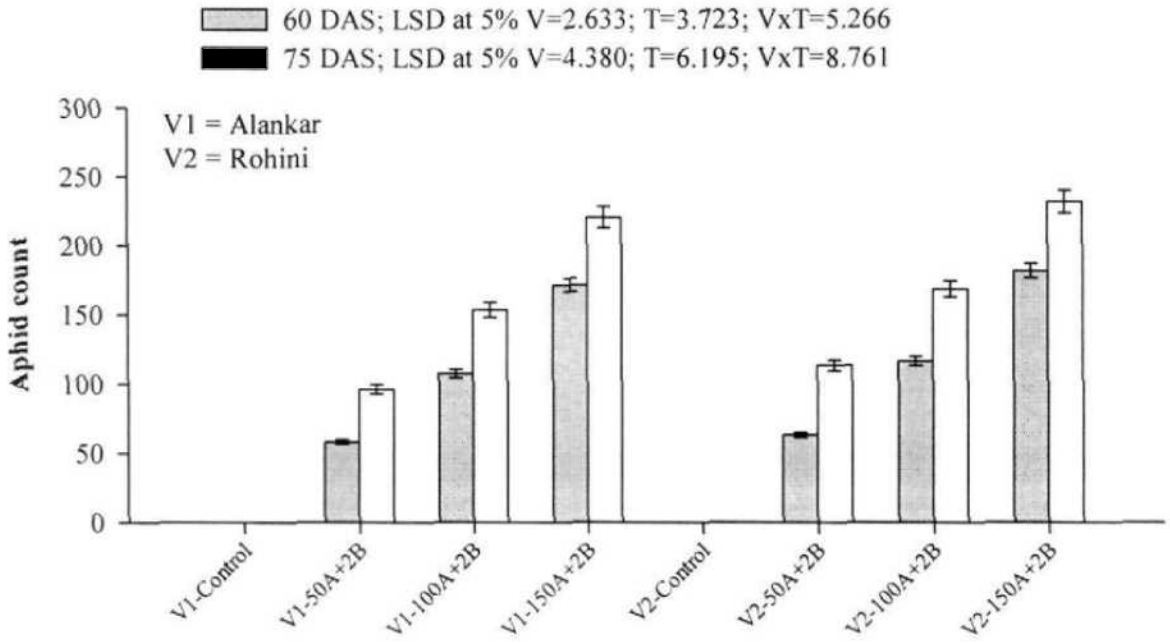


Fig. 73. Responses of aphid populations on *Brassica juncea* cvs. Alankar and Rohini exposed at 45 DAS to varying level of aphid infestations (0, 50, 100, 150 aphid per plant) and successive predation of aphids (5 days after) by beetle per plant at 60 and 75 DAS

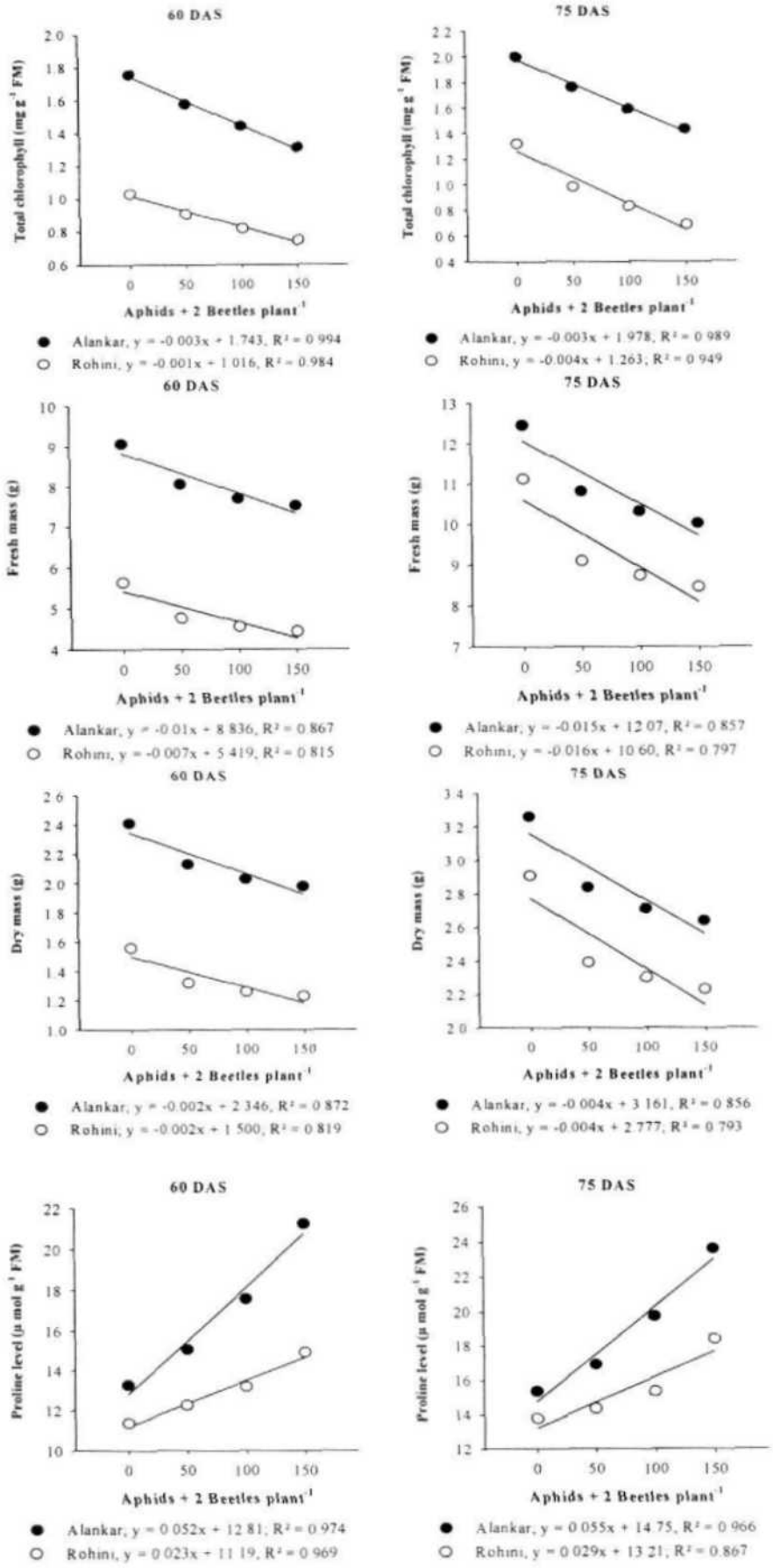


Fig.LR-V. Linear Regression line with equation and squired correlation coefficient between various growth parameters and aphid infestation level (under predation of 2 beetles) in selected cultivars at 60 and 75 DAS stages.

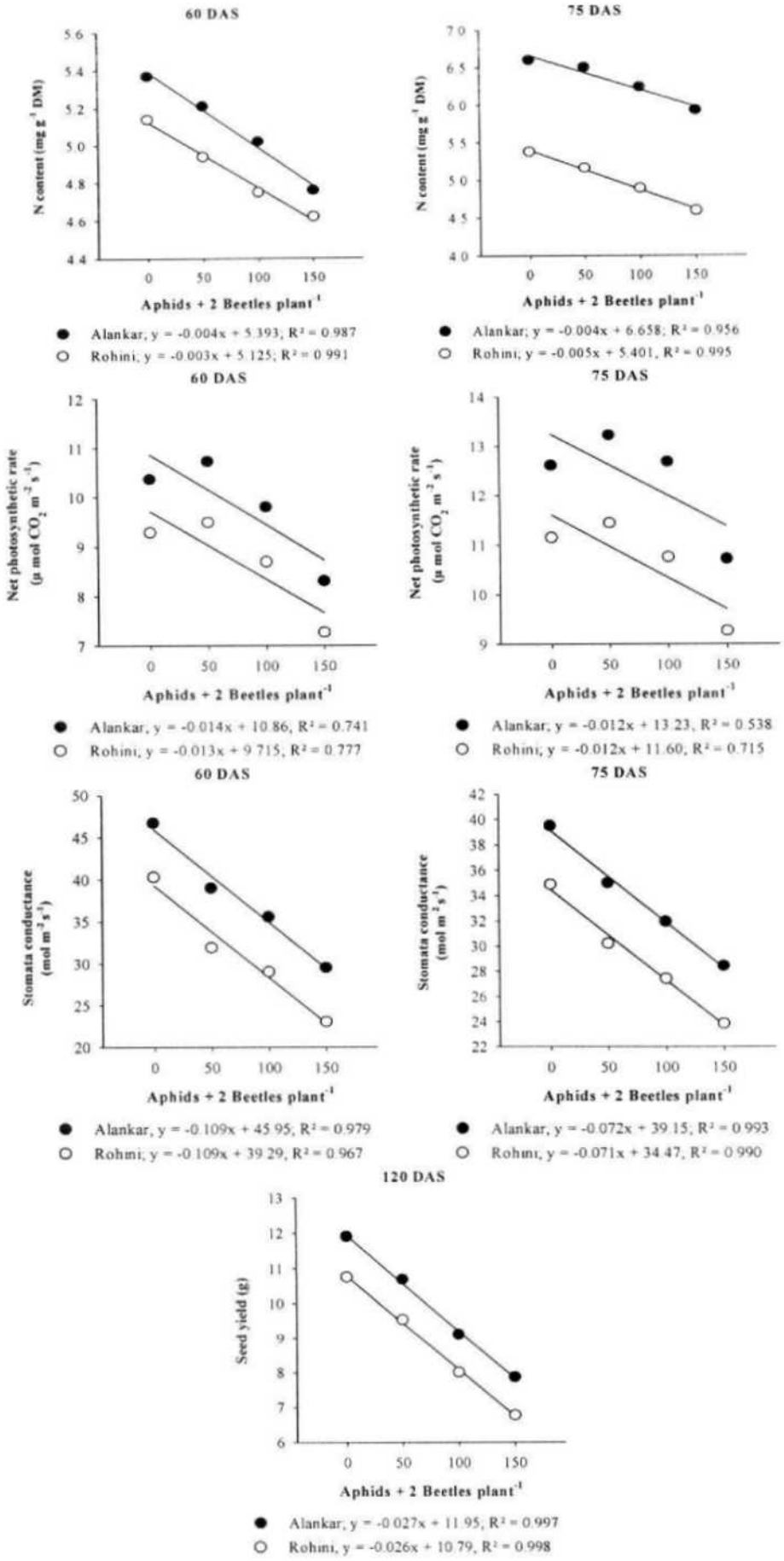


Fig. LR-VI. Linear Regression line with equation and squired correlation coefficient between various biochemical and yield parameters vs. aphid infestation level (under predation of 2 beetles) in selected cultivars at 60 and 75 DAS stages.

Correlation coefficients and regression analysis

The correlation coefficients between selected growth parameters and varying levels of aphid (under predation of 2 beetles per plant) were determined and regression lines plotted. The degrees of correlation were strong and negative. The highest degree of correlation was observed between N content and aphid population (LR-V) as well as between seed yield and aphid counts (Fig- LR- VI).

Experiment 4:

Effects of concentrations of jasmonic acid on mustard

To study the effects of jasmonic acid (JA) in plant defenses and find out the effective concentrations of JA to be used in the following Experiment 5, both the selected cultivars were treated with three varying concentrations of JA and responses were studied. Significant increase in the shoot and root length of cultivar Alankar was noted in response to the treatment with 0.5, 1.0, and 1.5 mM of JA. The best results were obtained on treatment with 1.0 mM of JA. There was no significant change in the shoot and root length of cv. Rohini treated with JA (Fig. 74, 75). The leaf number and area of both the cultivars did not show any significant change in response to JA at 60 DAS growth stage. But at late stage (75 DAS); JA exposure enhanced the leaf number to some extent in both the selected cultivars (Fig. 76, 77).

The JA treatments increased fresh and dry mass significantly in cv. Alankar and Rohini at both the growth stages 60 and 75 DAS (Fig. 78, 79). The chlorophyll content (a, b, and total) also increased on treatment with JA at both the growth stages (Fig. 80-82). Relatively higher impact of JA treatment was noted on carotenoid content in both the cvs. at 60 and 75 DAS (Fig. 83). The treatment with 1.0 and 1.5 mM of JA enhanced the protein content significantly in cv. Alankar at 60 and 75 DAS (Fig. 84). But, in cv. Rohini the significant increase of protein was noted only on treatment with 1.0 mM JA (Fig. 84). The phenol content in cv. Alankar and Rohini increased to relatively higher extent on treatment with 1.0 mM JA at 60 and 75 DAS (Fig. 85) On the basis of per cent variation, the proline content increased significantly on treatment with JA in both the selected cvs. (Alankar and Rohini). The increase in proline levels in both the cvs. corresponded with the concentration of JA (Fig. 86). The JA treatment enhanced nitrogen (N) content in both the cultivars (Alankar and Rohini). The highest increase nitrogen, phosphorus and potassium content were noted

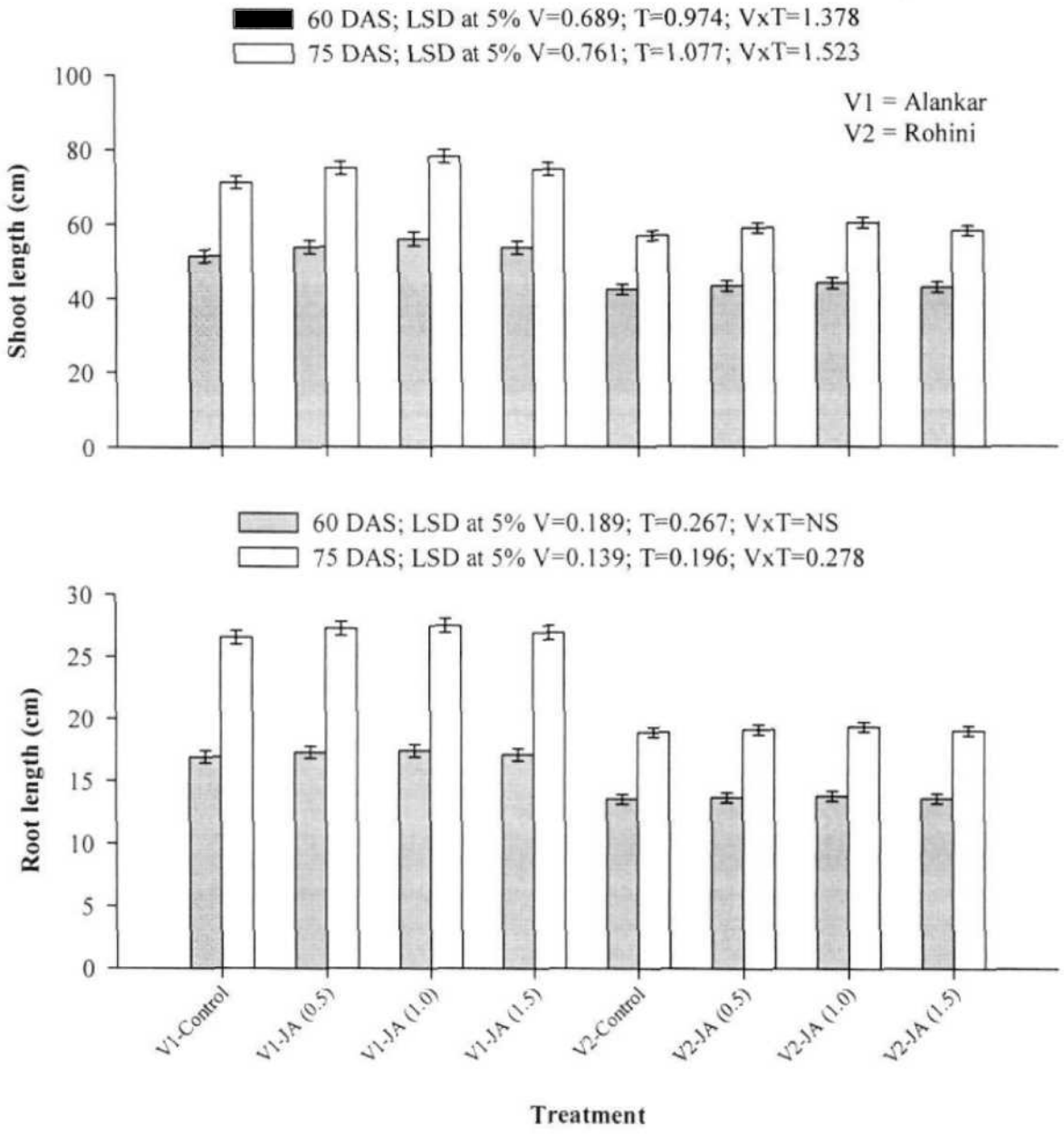


Fig 74, 75 Effect of jasmonic acid (0.5, 1.0 and 1.5 mM) on shoot and root length (cm) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

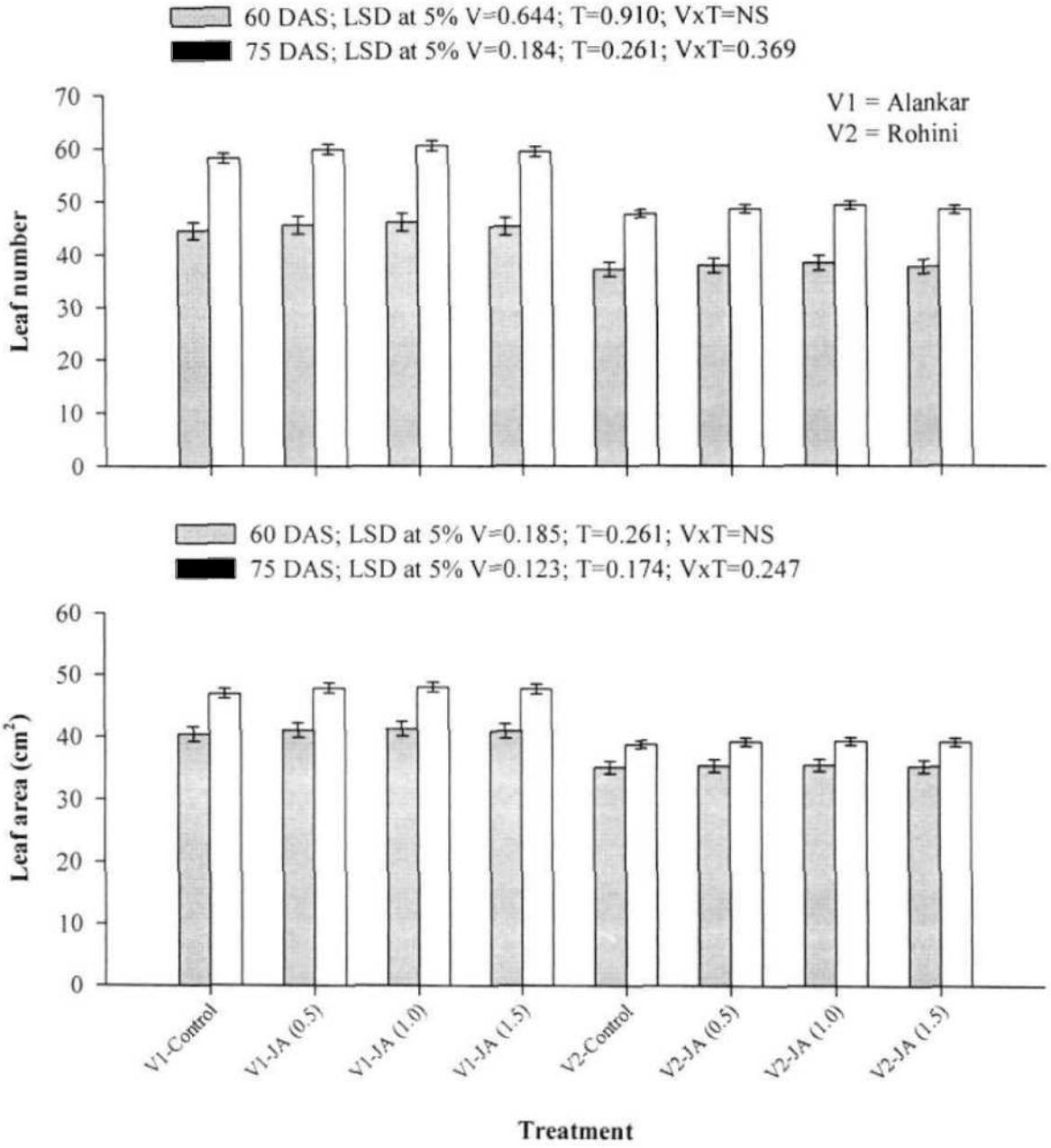


Fig. 76 ,77. Effect of jasmonic acid (0.5, 1.0 and 1.5 mM) on leaf number and area (cm^2) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

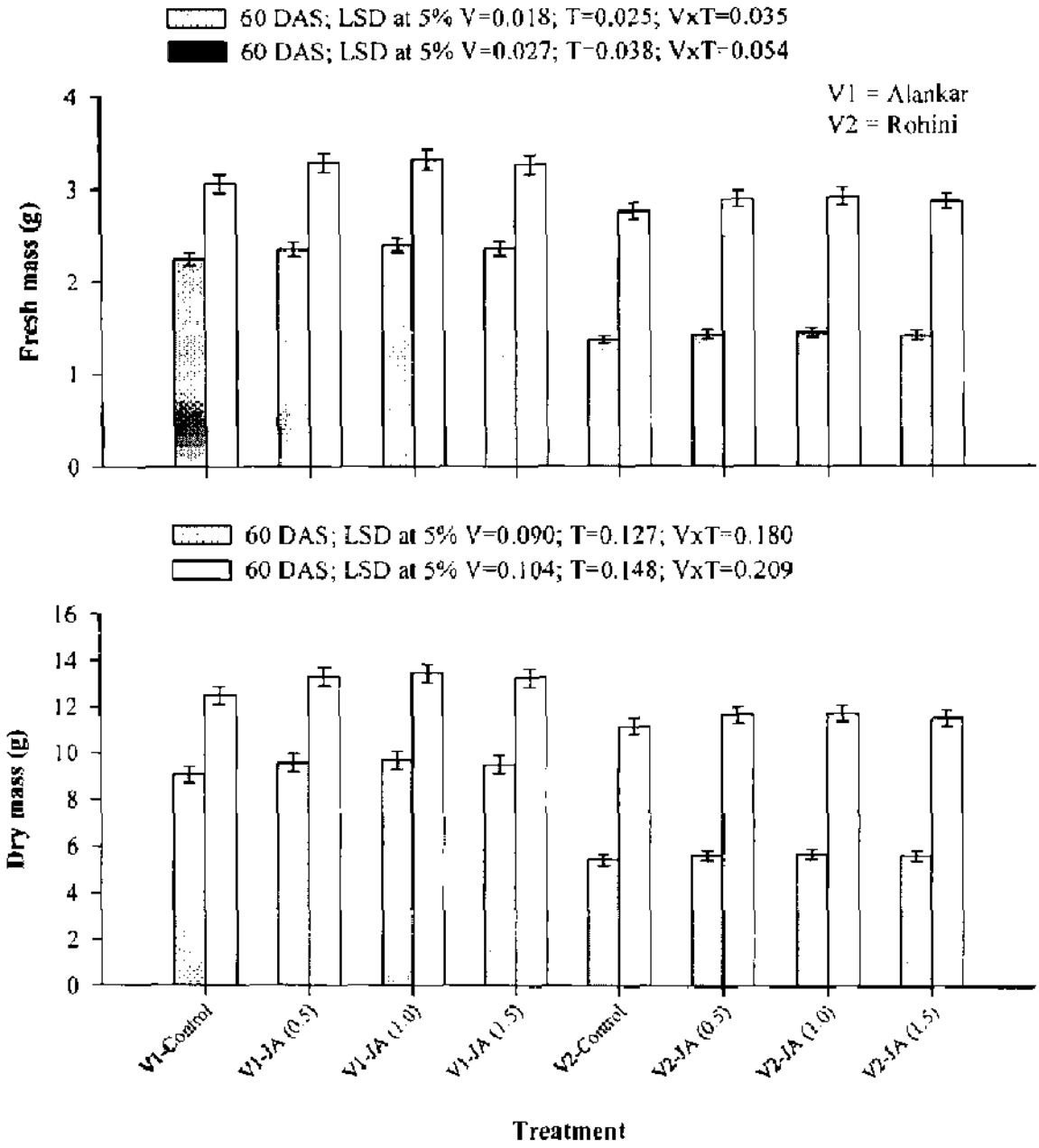


Fig. 78, 79. Effect of jasmonic acid (0.5, 1.0 and 1.5 mM) on fresh and dry mass (g) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

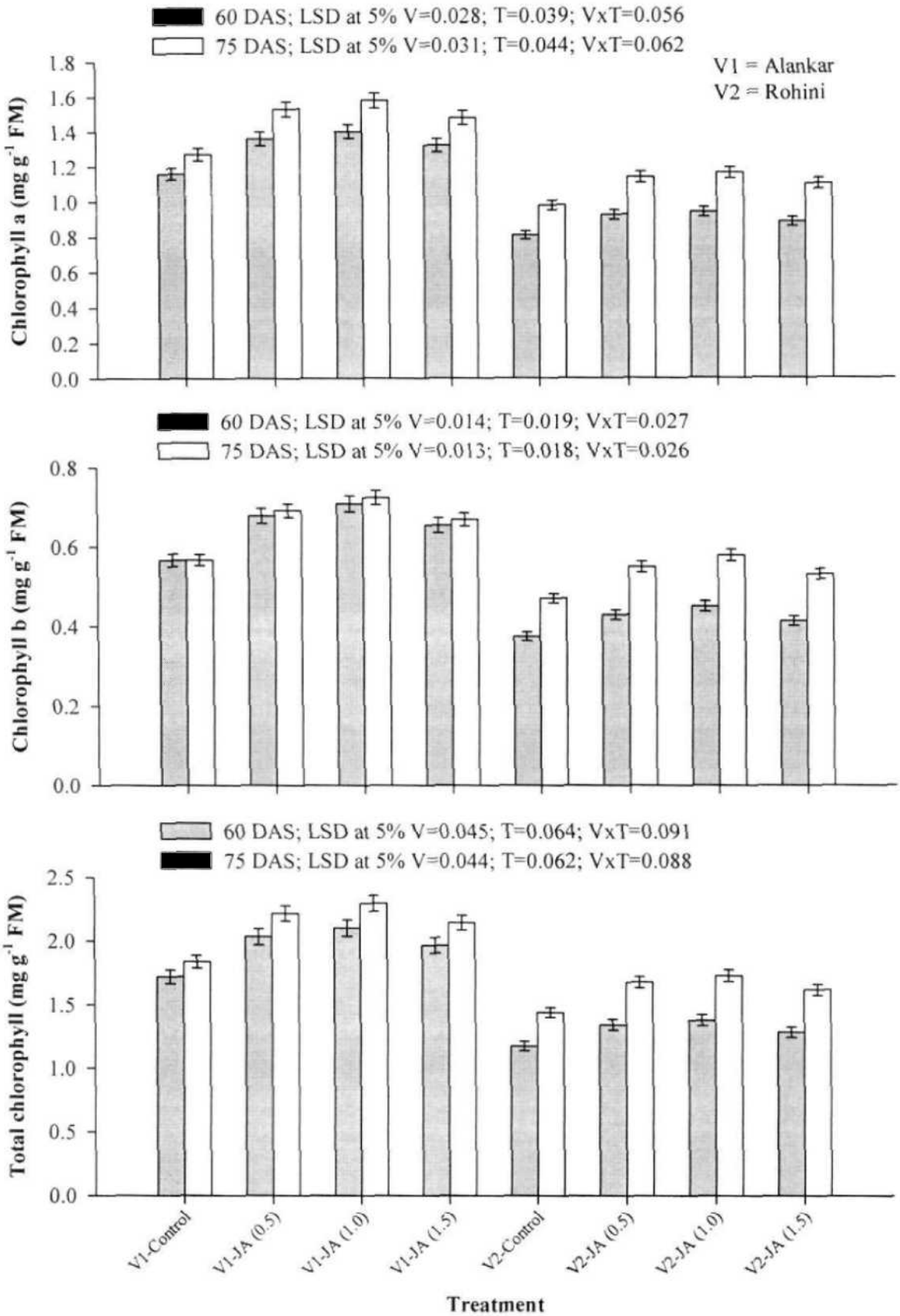


Fig. 80, 81, 82. Effect of jasmonic acid (0.5, 1.0 and 1.5 mM) on chlorophyll a and b and total chlorophyll (mg g^{-1} FM) level of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

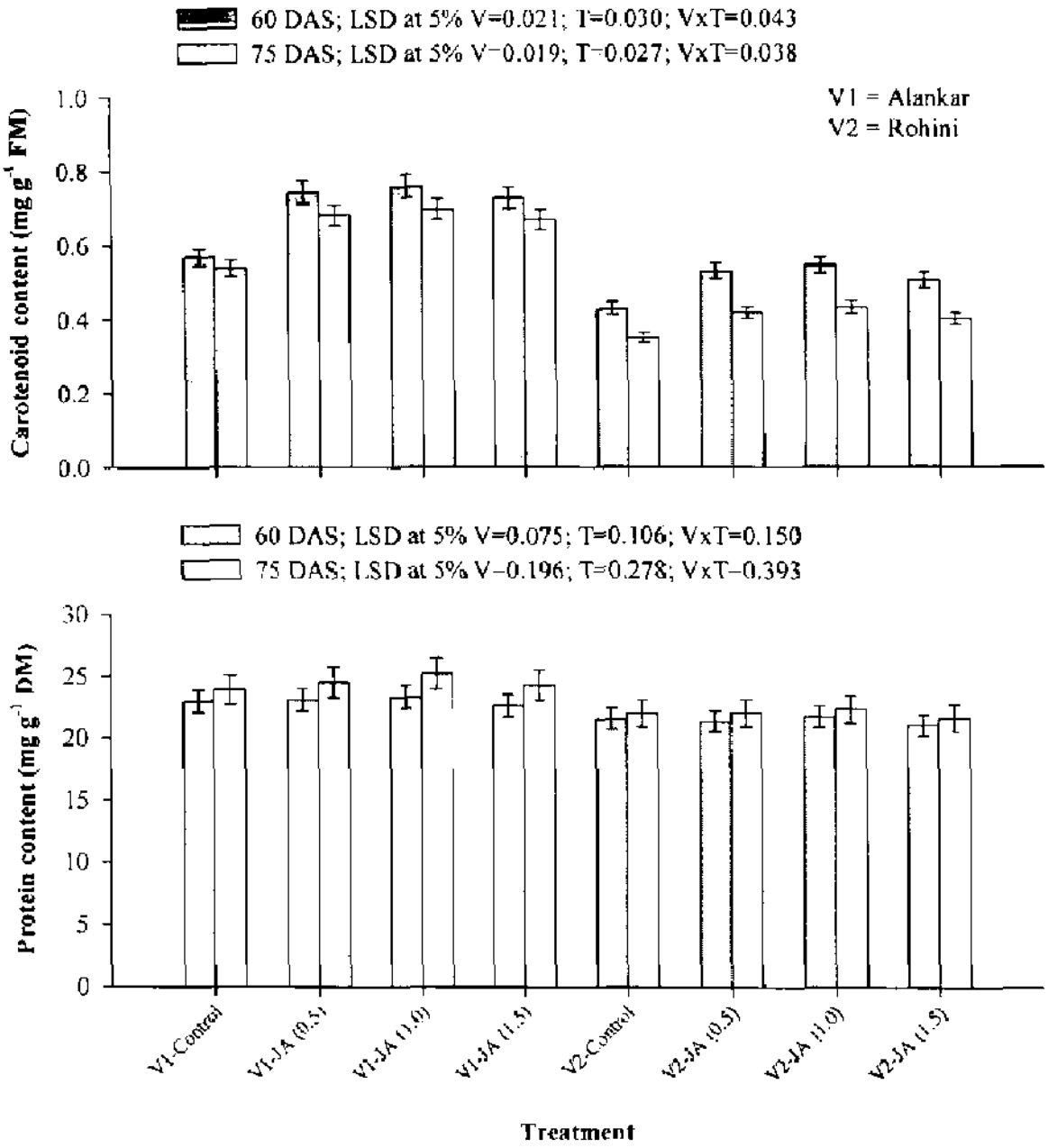


Fig. 83, 84. Effect of jasmonic acid (0.5, 1.0 and 1.5 mM) on carotenoid level (mg g^{-1} FM) and protein content (mg g^{-1} DM) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

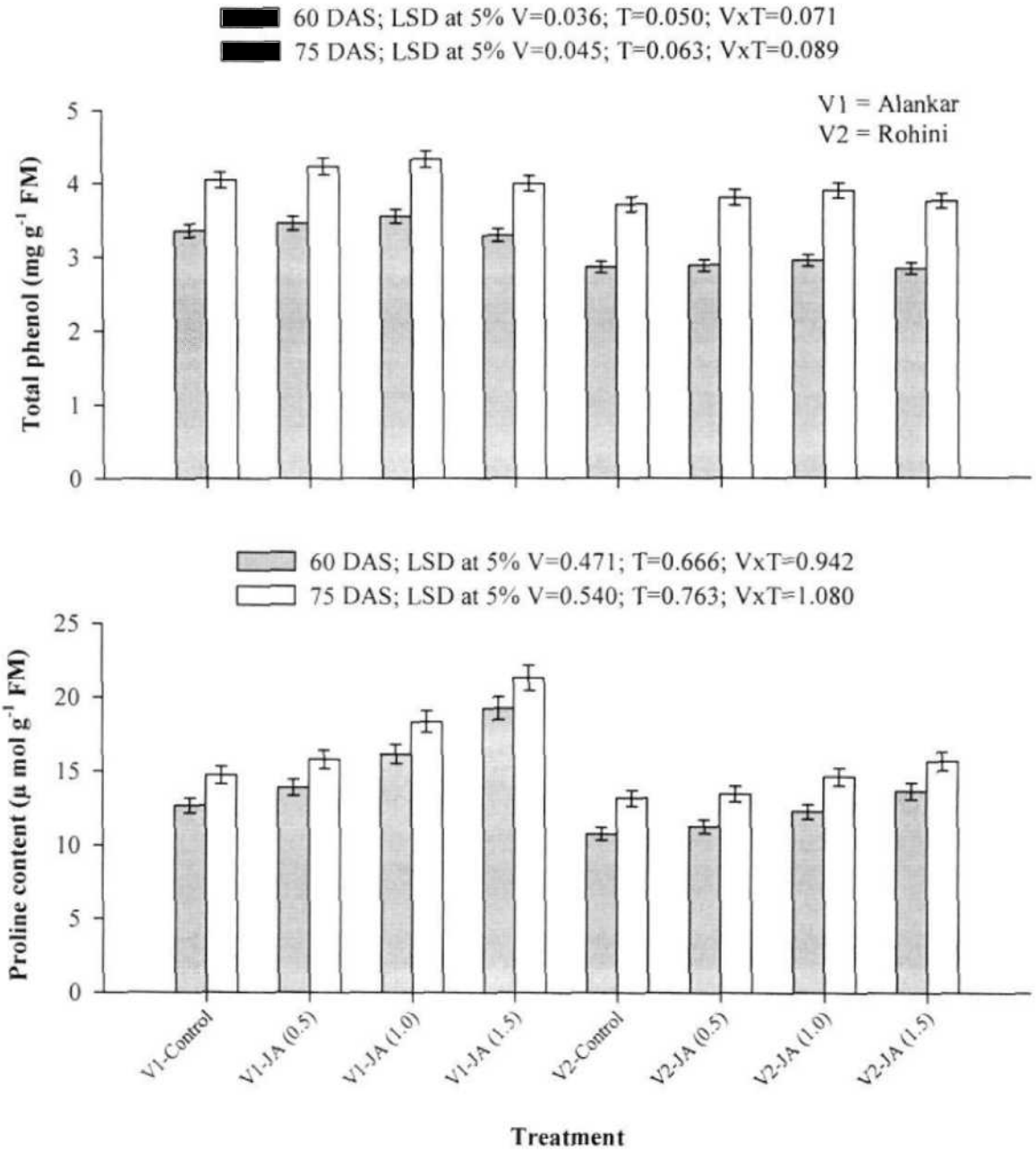


Fig 85, 86. Effect of jasmonic acid (0.5, 1.0 and 1.5) on total phenol and proline content (µ mol g^{-1} FM of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

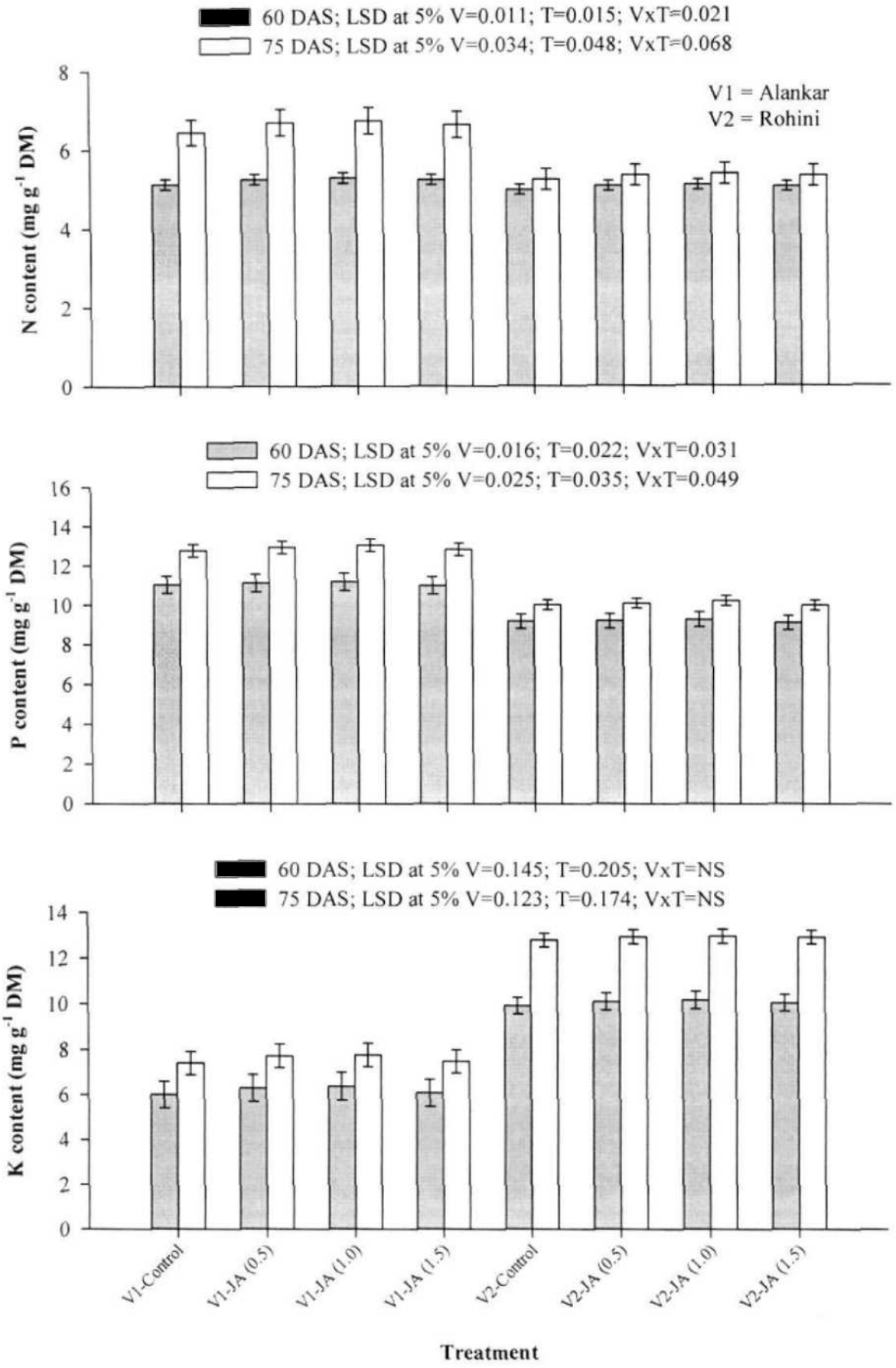


Fig- 87, 88, 89 Effect of jasmonic acid (0.5, 1.0 and 1.5 mM) on N, P and K content (mg g^{-1} DM) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

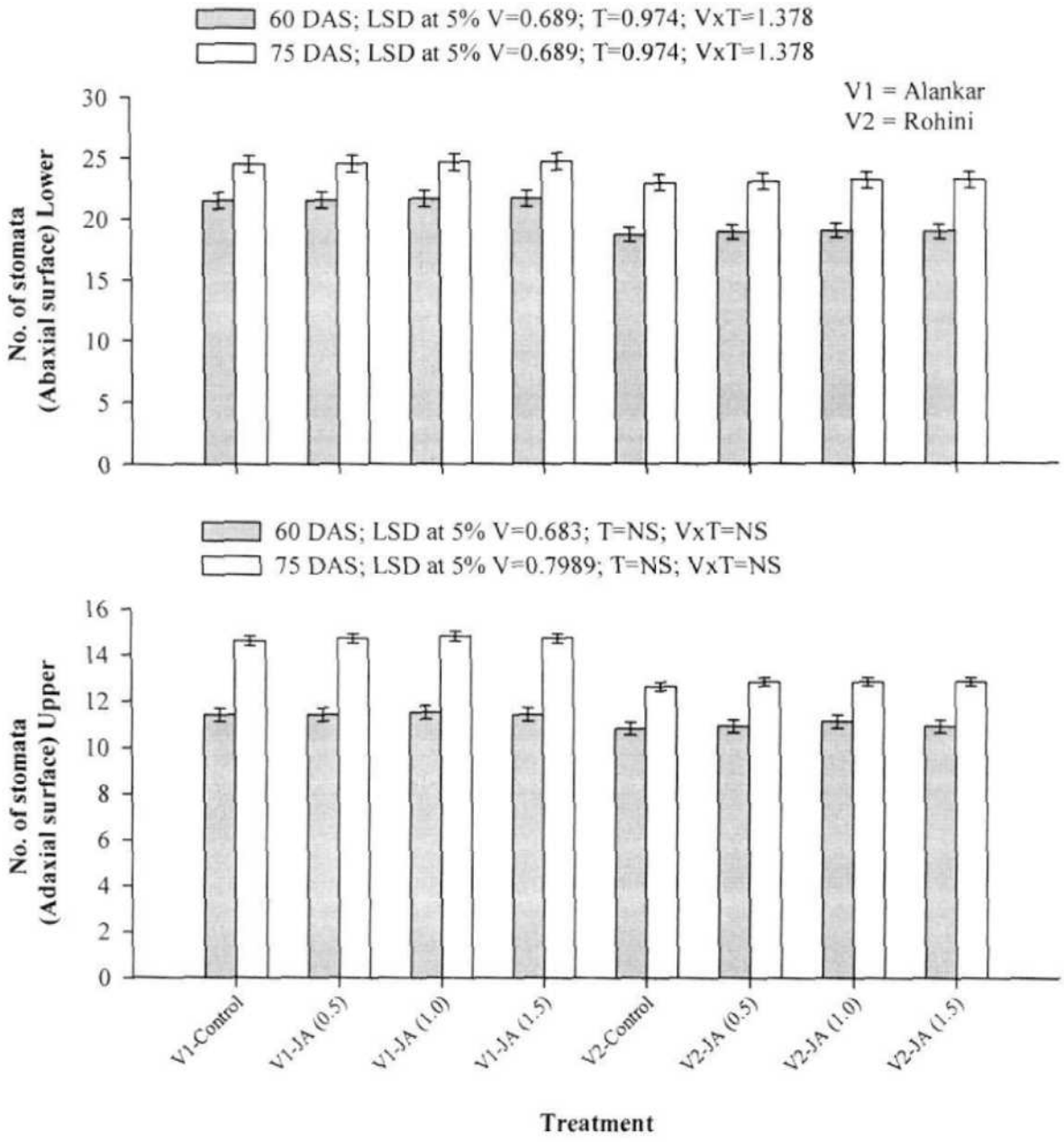


Fig. 90, 91. Effect of jasmonic acid (0.5, 1.0 and 1.5 mM) on number of stomata on abaxial and adaxial leaf surface of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

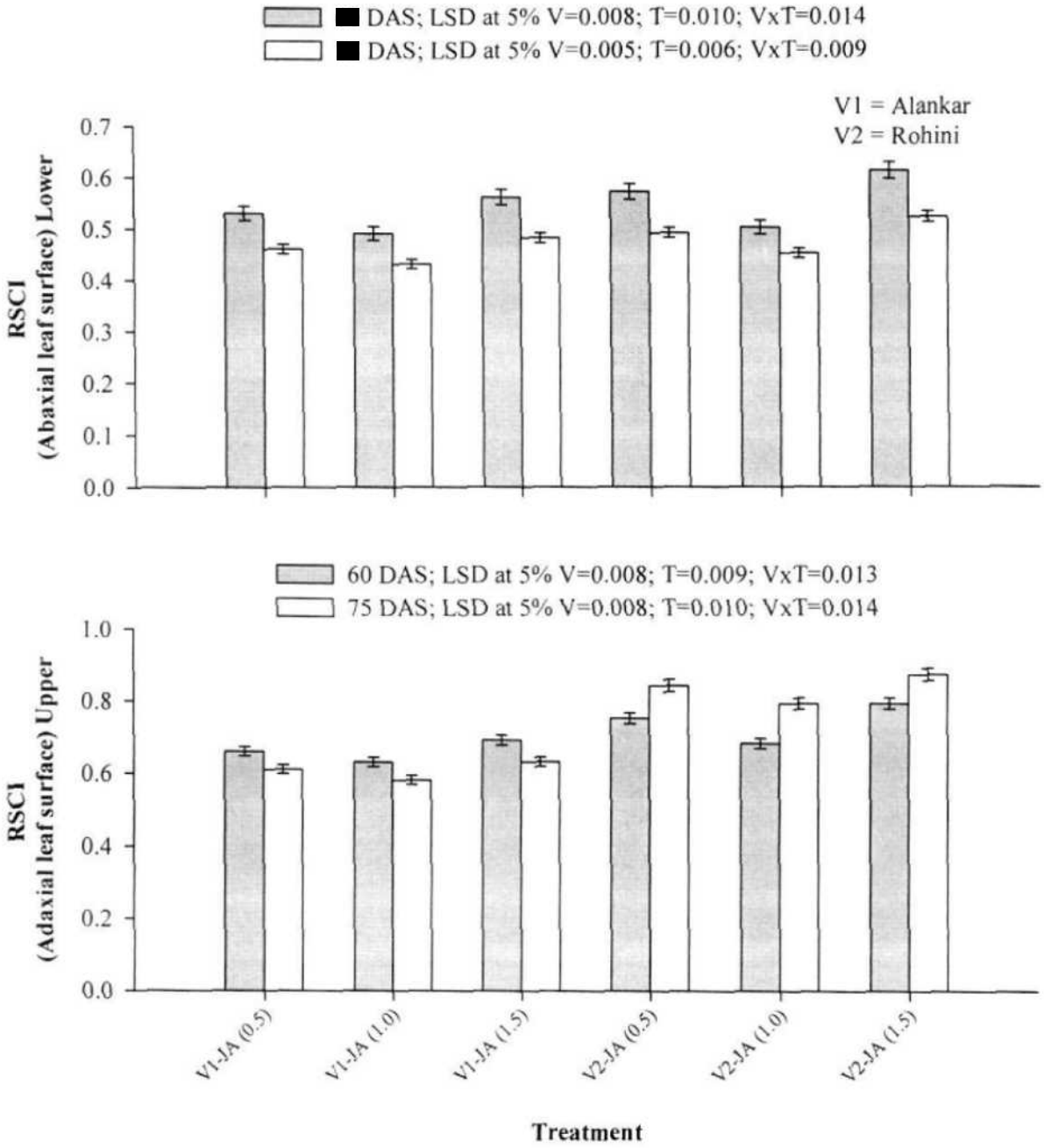


Fig. 92, 93 Effect of jasmonic acid (0.5, 1.0 and 1.5 mM) on relative stomatal closure index (RSCI) of abaxial and adaxial surface of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

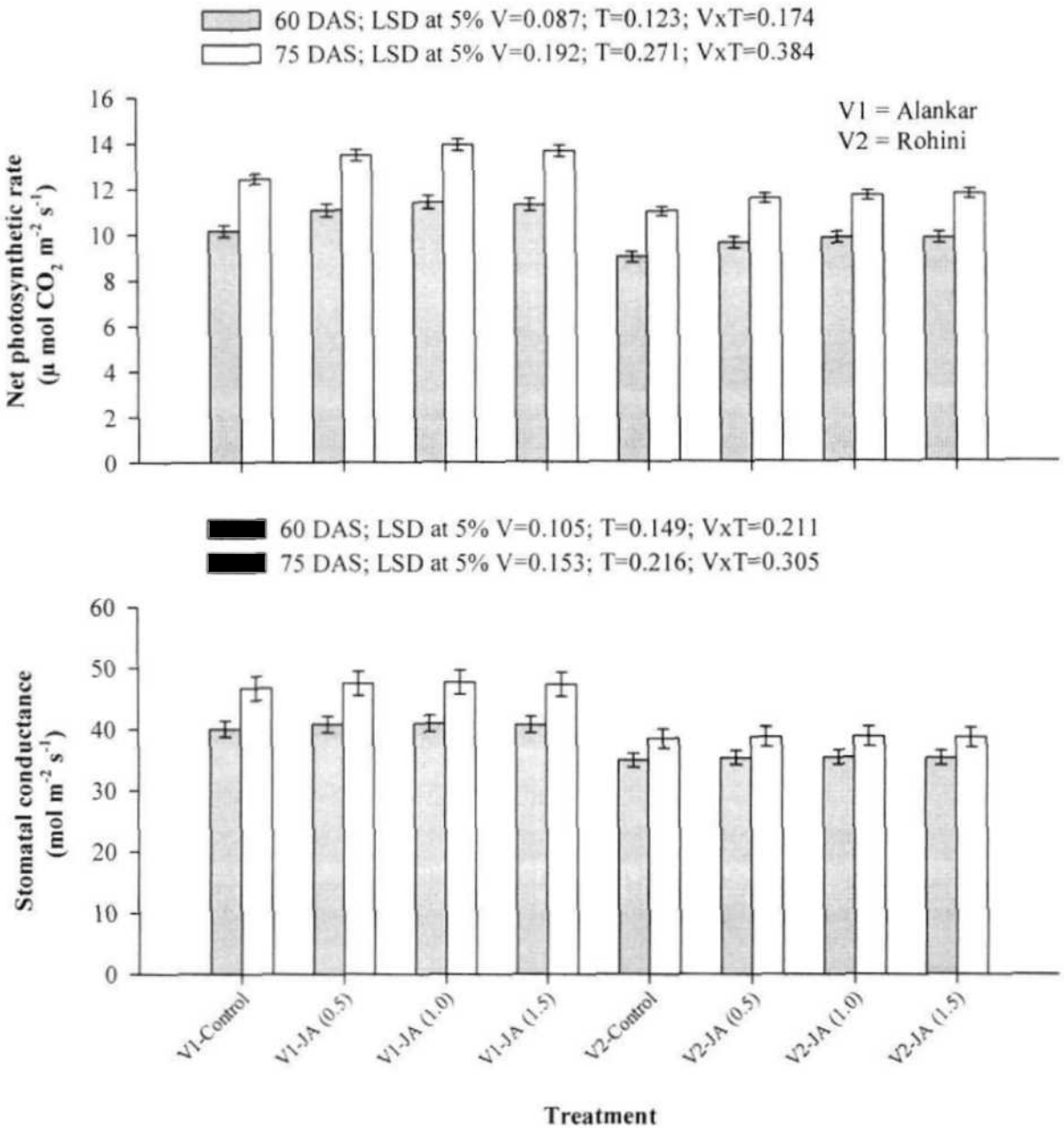


Fig. 94, 95 Effect of jasmonic acid (0.5, 1.0 and 1.5 mM) on net photosynthetic rate (P_N; μ mol CO_2 m^{-2} sec^{-1}) and stomatal conductance (g_s; mol m^{-2} sec^{-1}) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

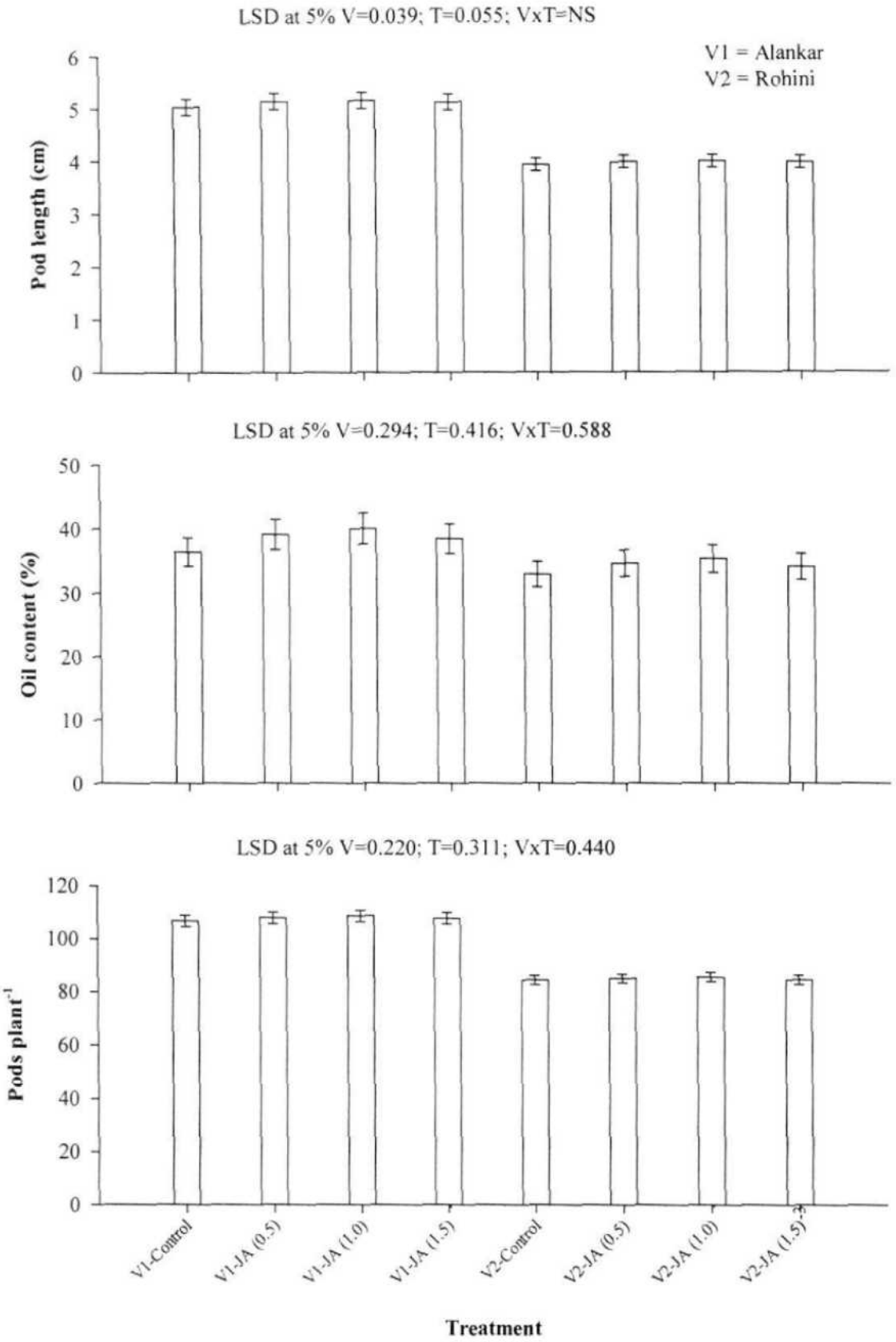


Fig. 96, 97, 98. Effect of jasmonic acid (0.5, 1.0 and 1.5 mM) on pod length (cm), oil content (%) and pod plant^{-1} of *Brassica juncea* cvs. Alankar and Rohini at harvest

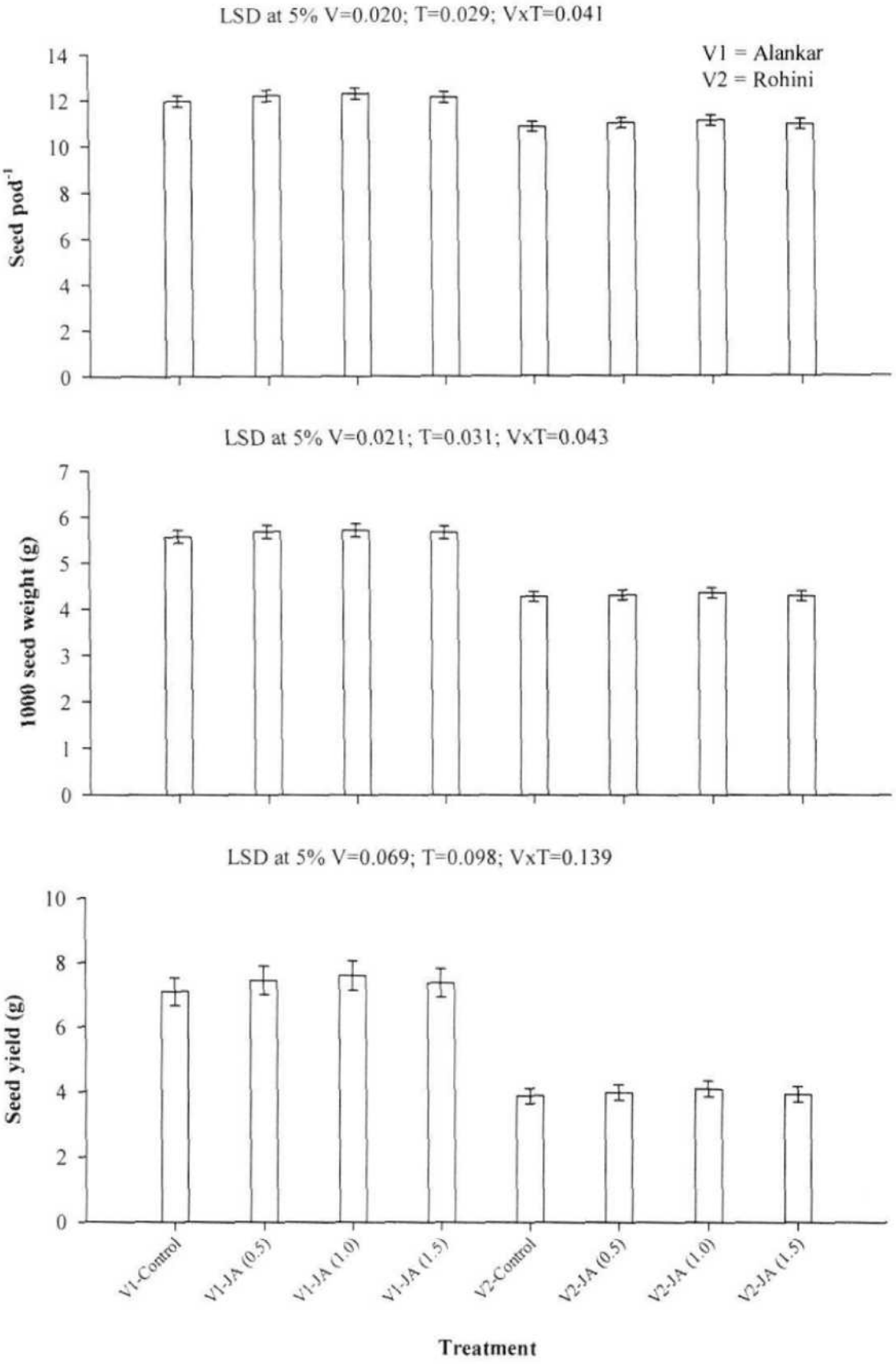


Fig. 99, 100, 101. Effect of jasmonic acid (0.5, 1.0 and 1.5 mM) on seed pod^{-1}, 1000 seed weight (g), seed yield (g) of *Brassica juncea* cvs. Alankar and Rohini at harvest.

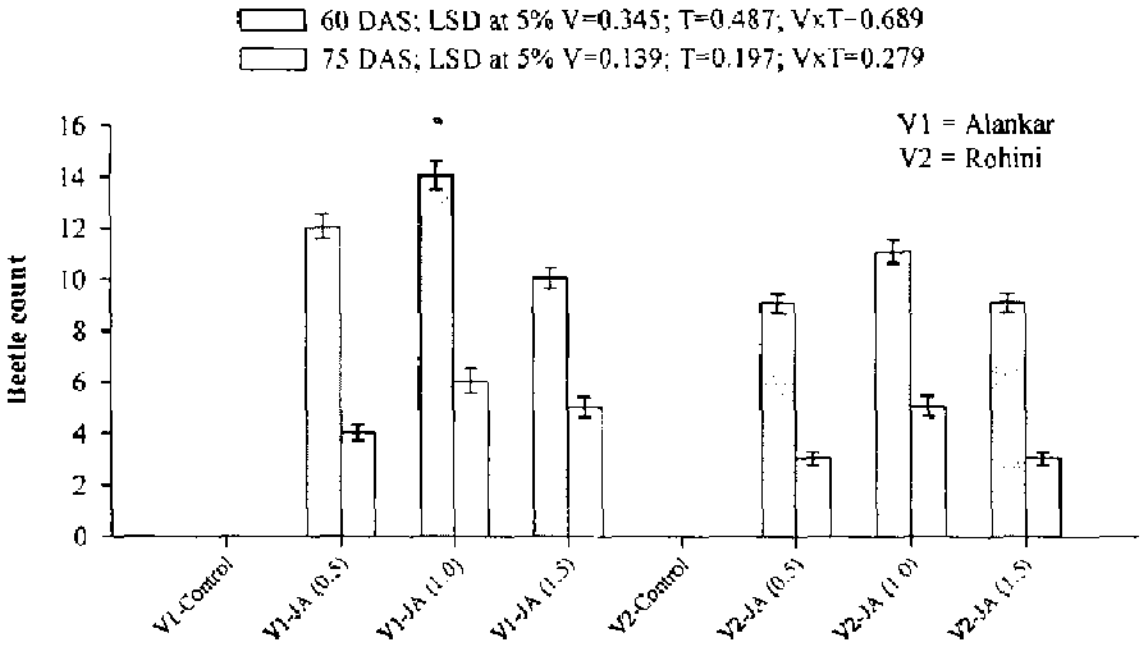


Fig.102 Effect of jasmonic acid (0.5, 1.0 and 1.5 mM) on number of beetles attracted on *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

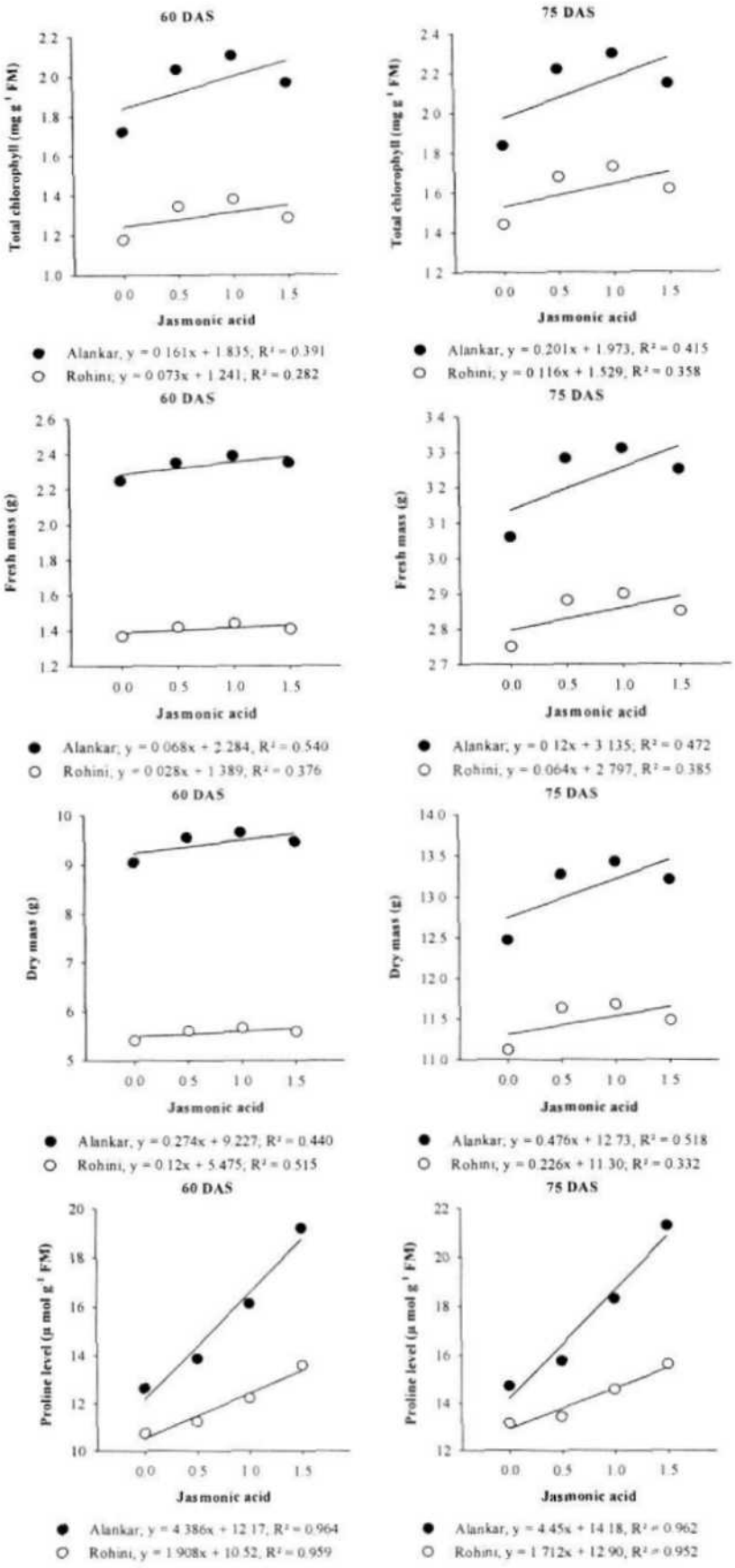


Fig. LR-VII. Linear Regression line with equation and squired correlation coefficient between various growth parameters and jasmonic acid (0.5, 1.0 or 1.5 mM) in selected cultivars at 60 and 75 DAS stages

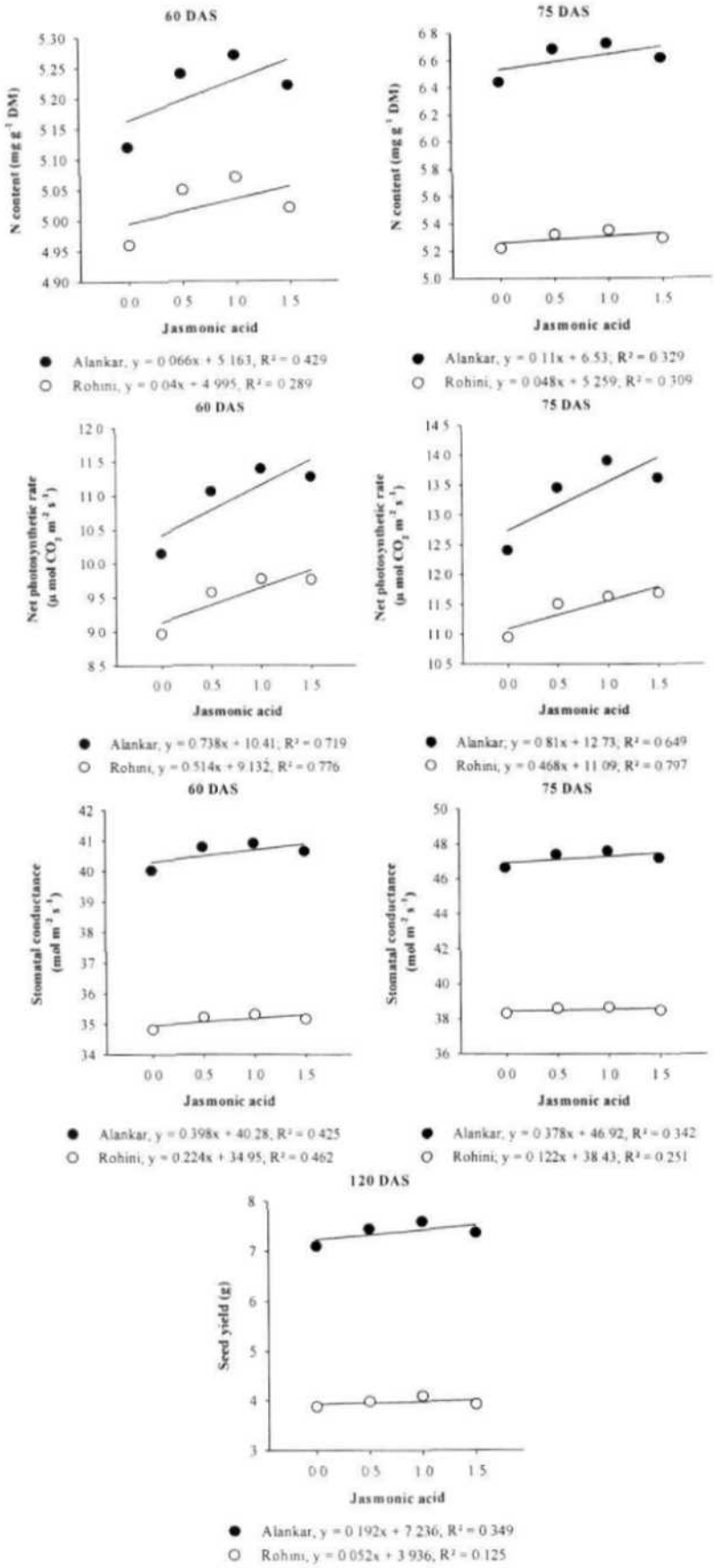


Fig.LR-VIII. Linear Regression line with equation and squired correlation coefficient between various biochemical and growth parameters vs. jasmonic acid (0.5, 1.0 or 1.5 mM) in selected cultivars at 60 and 75 DAS stages.

on treatment with 1.0 mM of JA (Fig. 87-89). The JA treatment did not affected stomatal frequency significantly on both the leaf surfaces in cvs. Alankar and Rohini (Fig. 90, 91). The JA treatment induced closure of stomata in both the cultivars as evident from the data on relative stomata closure index (Fig. 92, 93). The higher concentrations of JA (1.0 and 1.5 mM) induced maximum closure of stomata in both the cultivars of mustard (Fig. 92, 93). The JA treatment with 1.0 mM enhanced net rate of photosynthesis and gaseous exchange through stomata in both the cultivars (Fig. 94, 95). The JA treatment increased the yield attributes i.e. pod length (cm), oil content (%), pod per plant, seed per pod, 1000 seed mass and seed yield per plant in both the cultivars (Alankar and Rohini) at harvest stage (Fig. 96-101). Interestingly, JA treatment attracted *Coccinella septumpunctata* (a specialist predatory beetle of aphid). The largest number of beetles were attracted by JA treated Alankar than Rohini at early stage of growth than at the late growth stage (Fig. 102).

From the result of this experiment, it is evident that JA treatment increased the growth only marginally. The increase in growth parameters were in a lower limit indicating that the JA only reduced the plant stress. The JA had been helpful in signaling the predatory beetles and thus partially mimicked the injuries caused by aphid attack. Therefore, the JA was tried to simulate the responses of plant injury caused by herbivores and primed the defense response before aphid attack.

Correlation coefficients and regression analysis

The correlation coefficient between selected plant growth parameters and JA concentrations were weak in both the cultivars except the correlation between proline contents and JA levels (Fig. LR-VII and VIII) indicating that the proline level get enhanced in proportion to the JA spray (0.5, 1.0, 1.5 mM) (Fig.-LR VII and VIII). The photosynthetic rate and JA concentration also had strong and positive correlation ((Fig.- LR VII and VIII).

Experiment 5

Combined effects of jasmonic acid and aphid infestation

The effects of varying levels of aphid infestation (50, 100, 150 aphids per plant at 60 and 75 DAS) on pre JA treated (1.0 mM JA per plant at 45 DAS) plants of two selected cultivars cvs. Alankar and Rohini were studied. The responses of various growth attributes and changes in the physiological and biochemical parameters were

estimated at 60 and 75 DAS together with numbers of beetles signalled/attracted by the plants with the combined treatment of JA + aphid.

The data on effects of JA and varying numbers of aphids on shoot length and root length at 60 and 75 DAS are complied in Fig. 103, 104, respectively. The effect of JA (1.0 mM) together with 50 aphids increased the shoot length significantly in both the cultivars indicating a better stress management by JA. But larger aphid population (1.0 mM JA + 150 aphids) reduced the shoot length marginally at 60 DAS (Fig. 103). The impact of JA was defensive in nature in cv. Alankar treated with JA + 100 aphids at 75 DAS (Fig. 103). The trends of response of root growth were almost similar to that of shoot length at both the growth stages (Fig. 104).

The combination of JA (1.0 mM) and varying levels of aphids (50, 100, and 150) did not statistically affect the leaf number in cv. Alankar (Fig. 105) but cv. Rohini was susceptible to the aphid attack even when pre-treated with JA (Fig. 105). At late stage of growth (75 DAS) the leaf number in cv. Alankar reduced significantly only at higher level of JA + aphid infestation, but the leaf number in cultivar Rohini remained susceptible to various combinations of JA and aphid at this stage (Fig. 105). The leaf area in both the selected cultivars reduced only marginally on all JA + aphid combinations at both the growth stages (Fig. 106). The fresh and dry mass of both selected cultivars plant decreased significantly on treatment with JA + varying aphid combinations at 60 and 75 DAS (Fig. 107, 108).

The combination of JA (1.0 mM) and varying levels of aphid reduced chlorophyll content (chlorophyll a, b and total) in both the cultivars Alankar and Rohini (Fig. 109-111). The reductions in chlorophyll contents were in proportion to the level of aphid (Fig. 109-111). However, the carotenoid content increased in the leaves of both the cultivars (Alankar and Rohini) on treatment with JA + varying levels of aphid at 60 and 75 DAS (Fig. 112). The increase in the carotenoid content was highest in both the cvs. on treatment with 1.0 mM JA + 50 aphids (Fig, 112).

The total protein content decreased in both the cultivars viz. Alankar and Rohini at 60 and 75 DAS on treatment with varying levels of 1.0 mM JA and aphids (Fig. 113). The phenol content also reduced in both the cultivars on treatment with JA + aphid. The highest decrease in phenol accumulation was recorded in both the cvs. on treatment with 1.0 mM of JA + 150 aphid (Fig. 114). The proline is a stress marker

and stress resisting amino acid. The accumulation of proline in response to the treatment with JA + aphid combination is shown in Fig. 115. The proline accumulation increased in both the cultivars Alankar and Rohini on a combined treatment with 1.0 mM JA + varying levels of aphids. The proline accumulation in both the cultivars consistently increased on treatment with 1.0 mM of JA + increasing number of aphids (Fig. 115).

The nitrogen, phosphorous content decreased in both the cultivars in proportion to the 1.0 mM JA + number of infesting aphids. But potassium content increased significantly in response to combine effect of JA and 150 aphids in both the cultivars at 60 DAS. At late stage there was no change in potassium content in cv. Alankar in response to various levels of JA + aphid (Fig. 116-118). The changes in stomatal frequency and relative stomata closure index (RSCI) were determined in both the cvs. in response to simulated and natural herbivory (JA + aphid combination). There was a marginal impact of varying numbers of JA + aphid on stomatal frequency. The stomatal frequency on both the leaf surfaces decreased in cvs. Alankar and Rohini (Fig. 119, 120). The RSCI increased significantly on combined treatment with 1.0 mM JA and varying levels of aphids (Fig, 121,122). In the present experiment, the closure of stomata on both the leaf surfaces was higher in cv. Rohini than in cv. Alankar (Fig, 121, 122).

The net photosynthetic rates increased in cvs. Alankar and Rohini on combined treatment with 1.0 mM JA and 50 aphids, but the stomatal conductance decreased. It may be due to reduced stomatal frequency and increased closure of stomata. But a combination of 1.0 mM JA + 150 aphids reduced the photosynthetic rate and stomatal conductance significantly in both the cultivars (Fig.123, 124).

The treatment with JA and varying levels of aphid combination significantly reduced pod elongation, seed mass and oil content in both the cultivars. The highest reductions in these parameters were recorded in responses to 1.0 mM and 150 aphids (Fig. 125-130).

Aphid and beetle demography

The aphid population was counted at 60 and 75 DAS in both the cultivars after treating with 1.0 mM of JA (at 45 DAS) and varying level of aphids (at 50 DAS). The aphid population reduced significantly in cv. Alankar on treatment with combinations

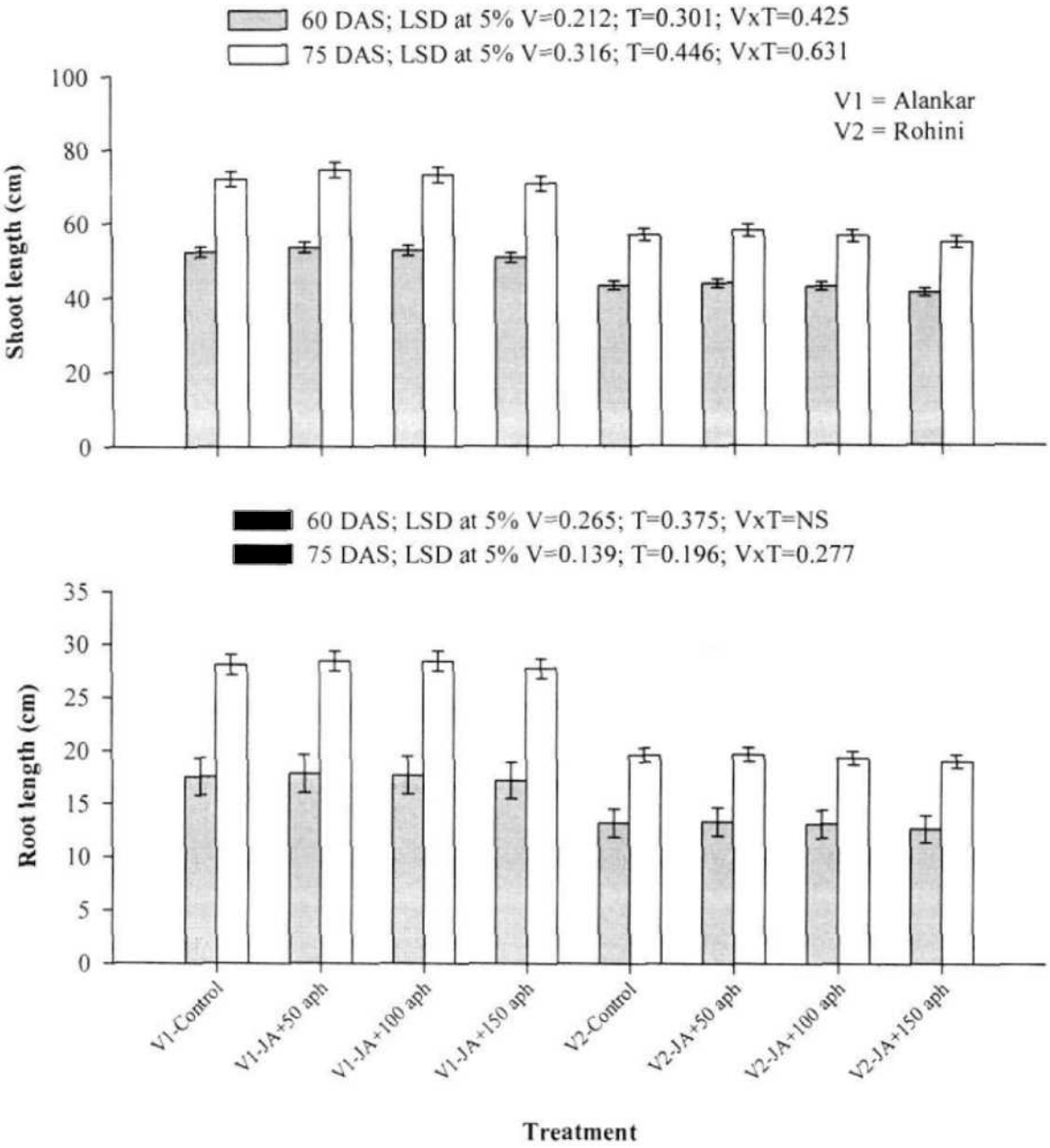


Fig. 103, 104. Combined effect of jasmonic acid (1.0 mM) and aphid infestation (50, 100 and 150 aphids per plant) on shoot and root length (cm) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

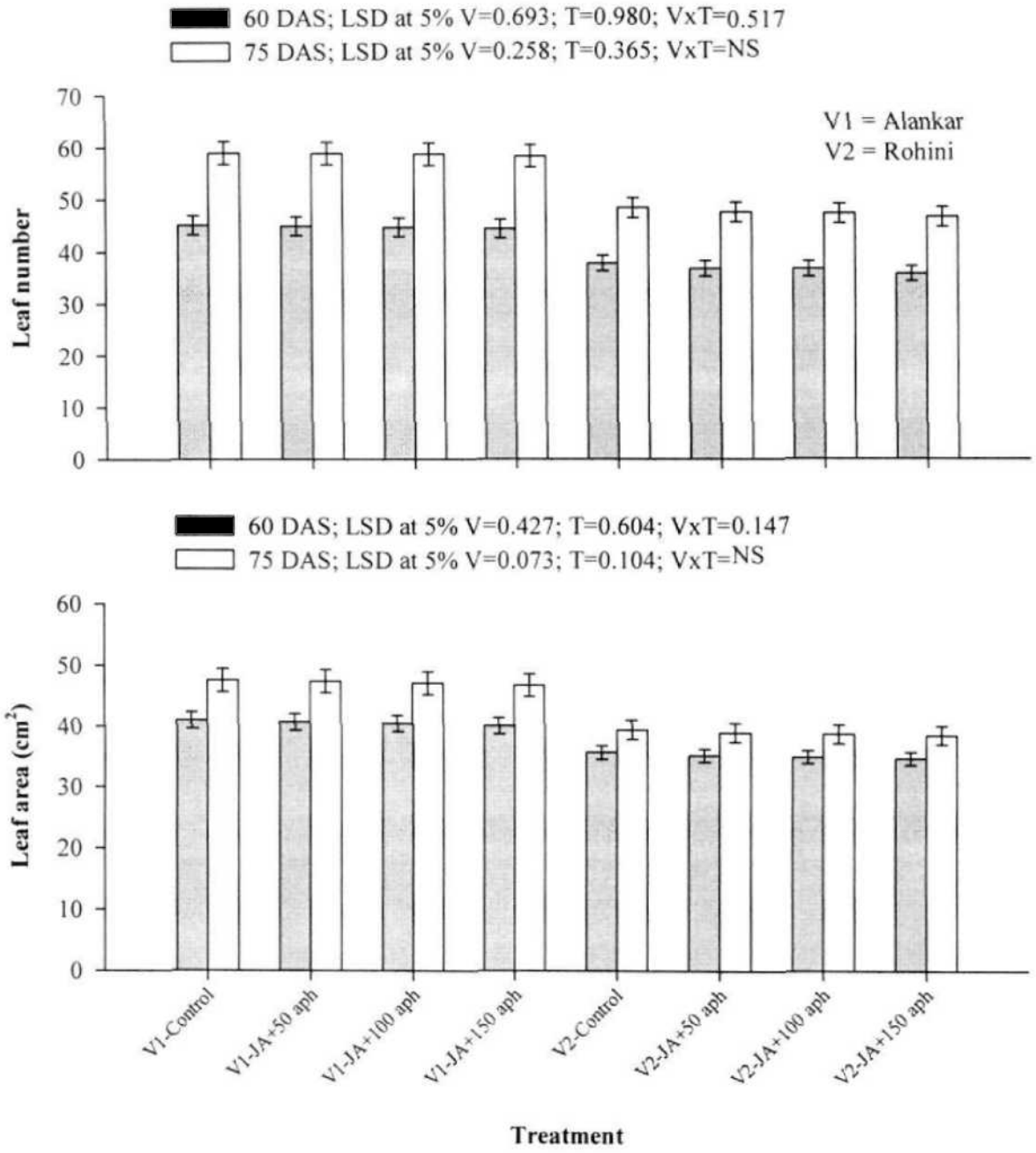


Fig. 105, 106. Combined effect of jasmonic acid (1.0 mM) and aphid infestation (50, 100 and 150 aphids per plant) on leaf number and leaf area (cm^2) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

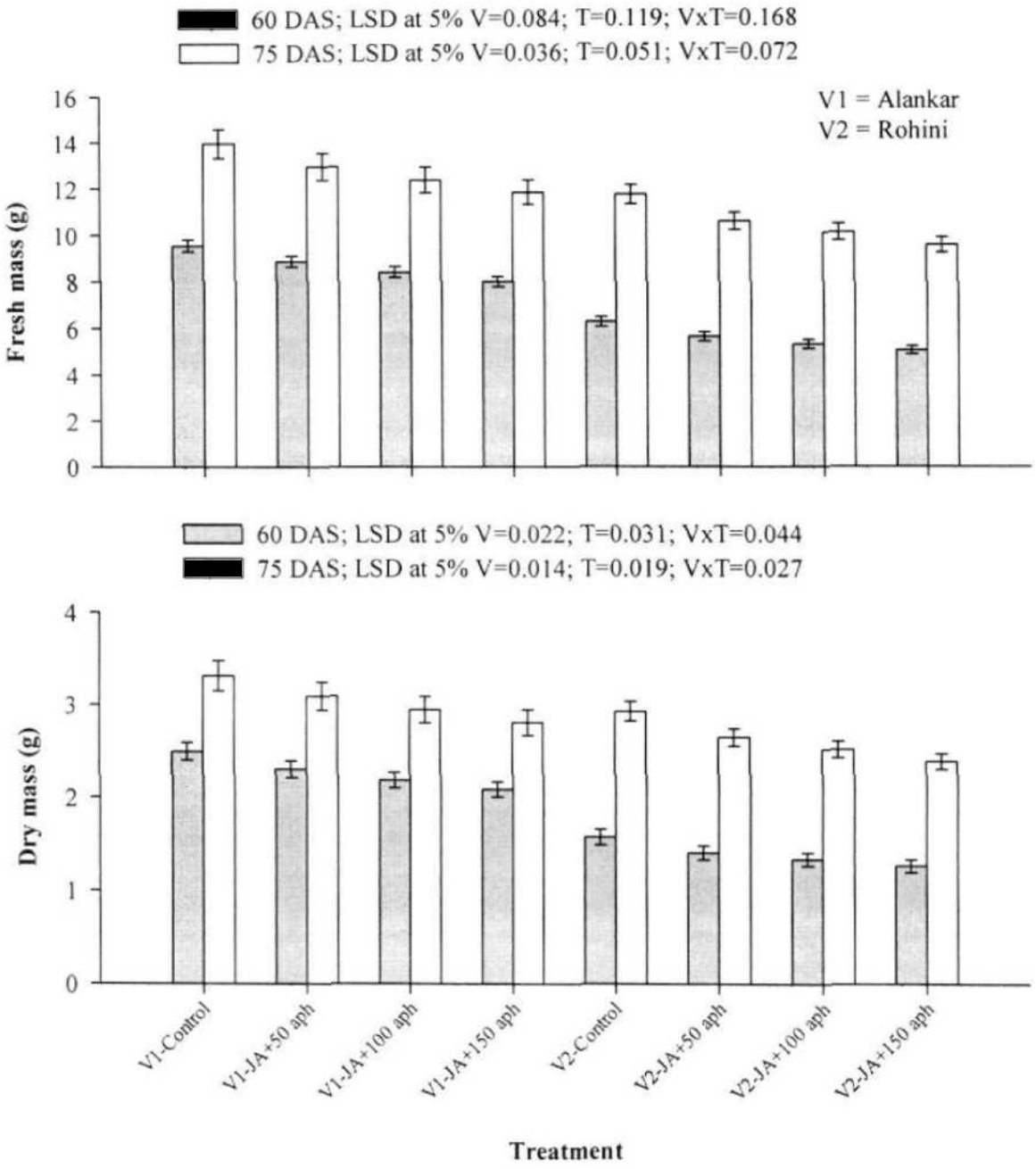


Fig. 107, 108. Combined effect of jasmonic acid (1.0 mM) and aphid infestation (50, 100 and 150 aphids per plant) on fresh and dry mass (g) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

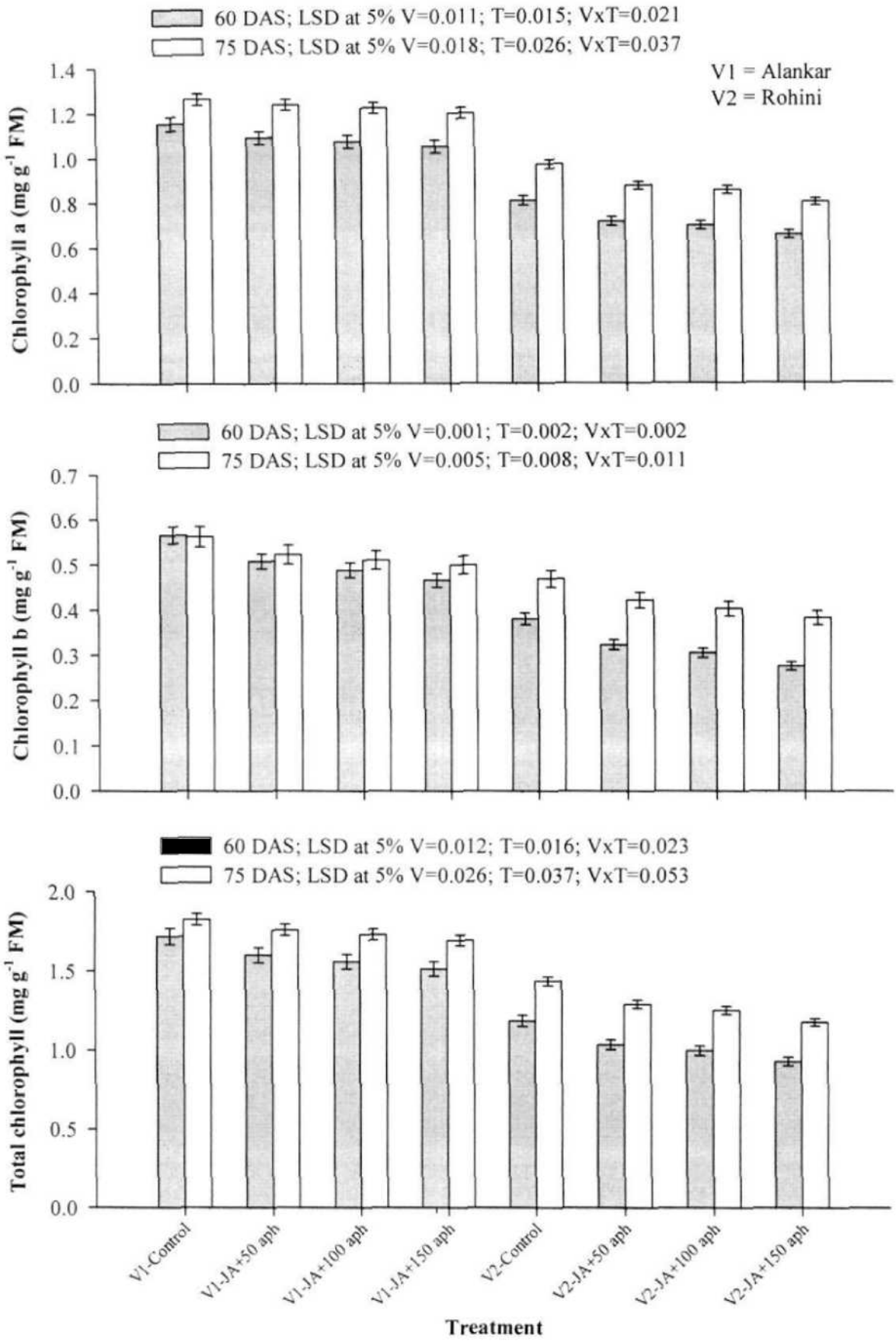


Fig. 109, 110. 111. Combined effect of jasmonic acid (1.0 mM) and aphid infestation (50, 100 and 150 aphids per plant) on chlorophyll a, b and total chlorophyll (mg g^{-1} FM) level of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

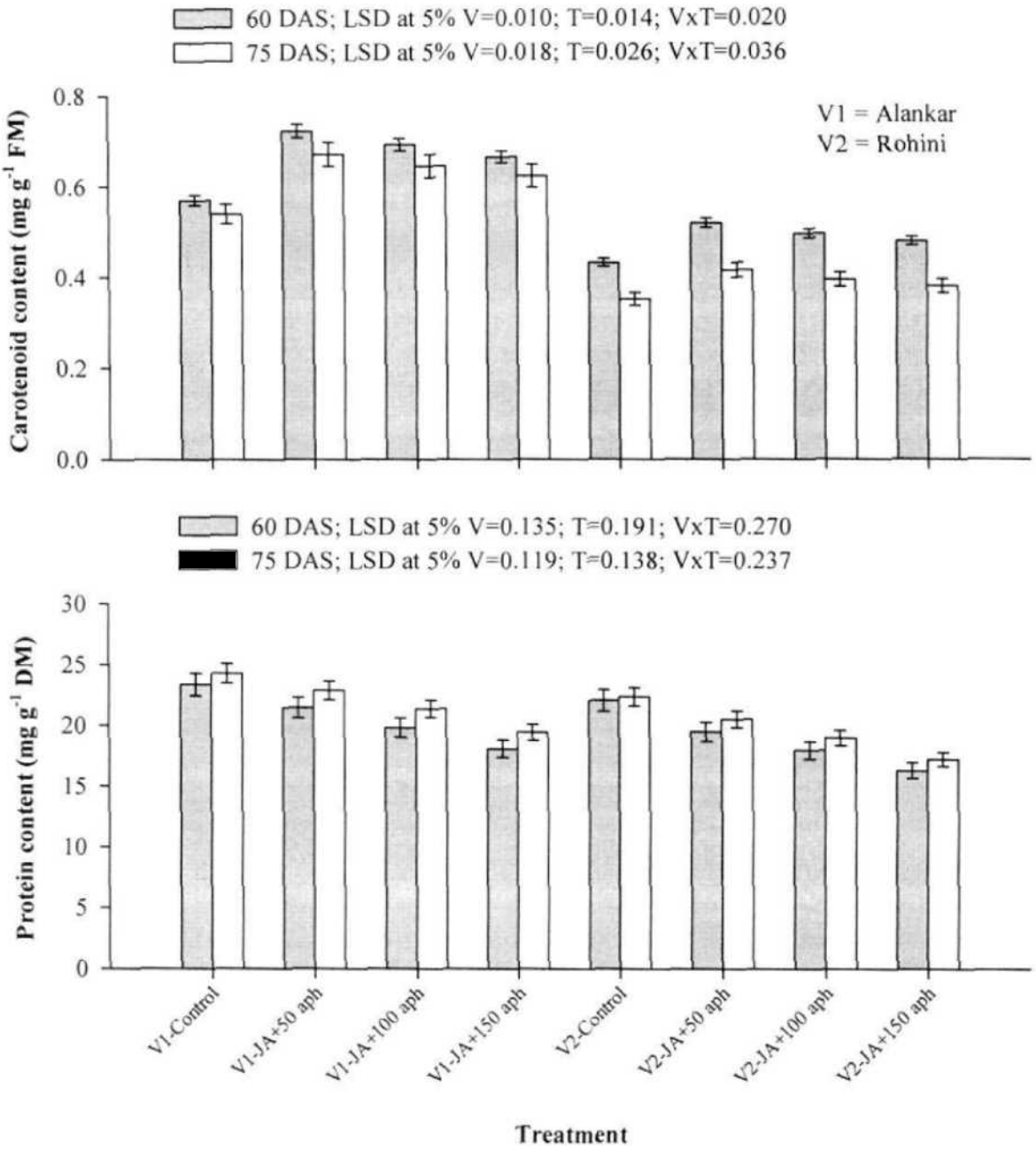


Fig. 112, 113. Combined effect of jasmonic acid (1.0 mM) and aphid infestation (50, 100 and 150 aphids per plant) on carotenoid level (mg g^{-1} FM) and protein content (mg g^{-1} DM) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

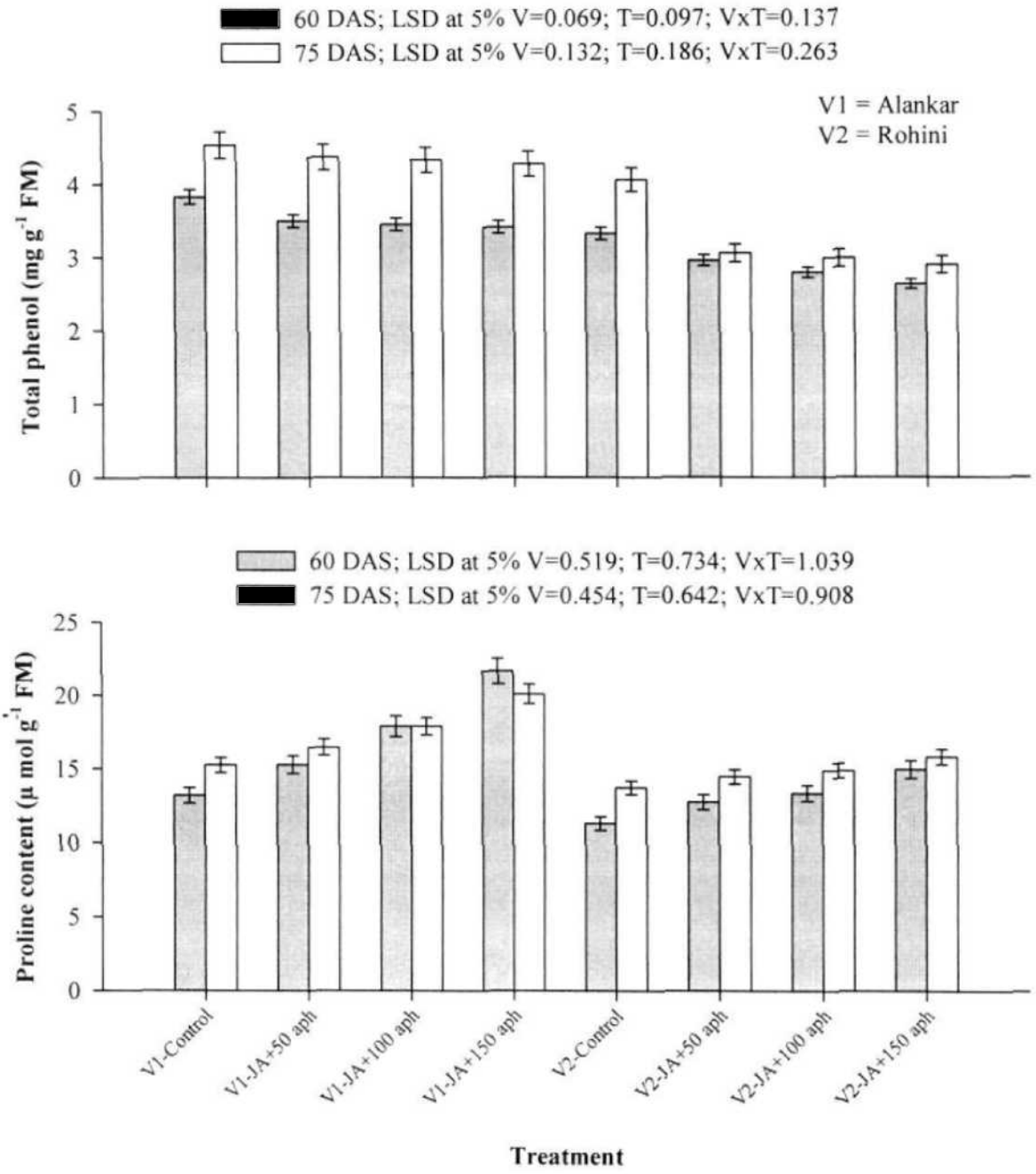


Fig. 114, 115. Combined effect of jasmonic acid (1.0 mM) and aphid infestation (50, 100 and 150 aphids per plant) on total phenol content (mg g^{-1} DM) and proline level (µ mol g^{-1} FM) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

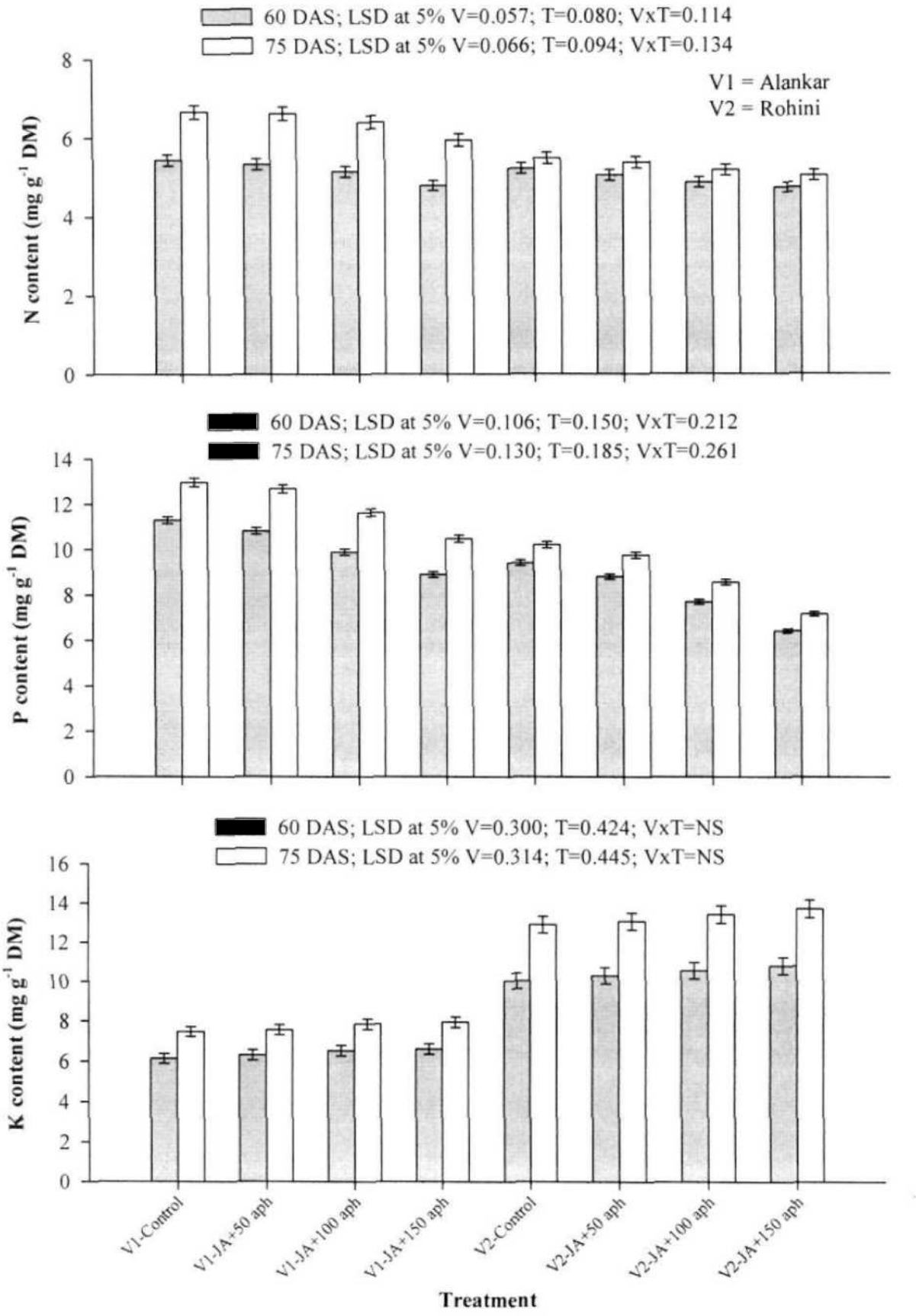


Fig. 116, 117, 118. Combined effect of jasmonic acid (1.0 mM) and aphid infestation (50, 100 and 150 aphids per plant) on N, P and K content (mg g^{-1} DM) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

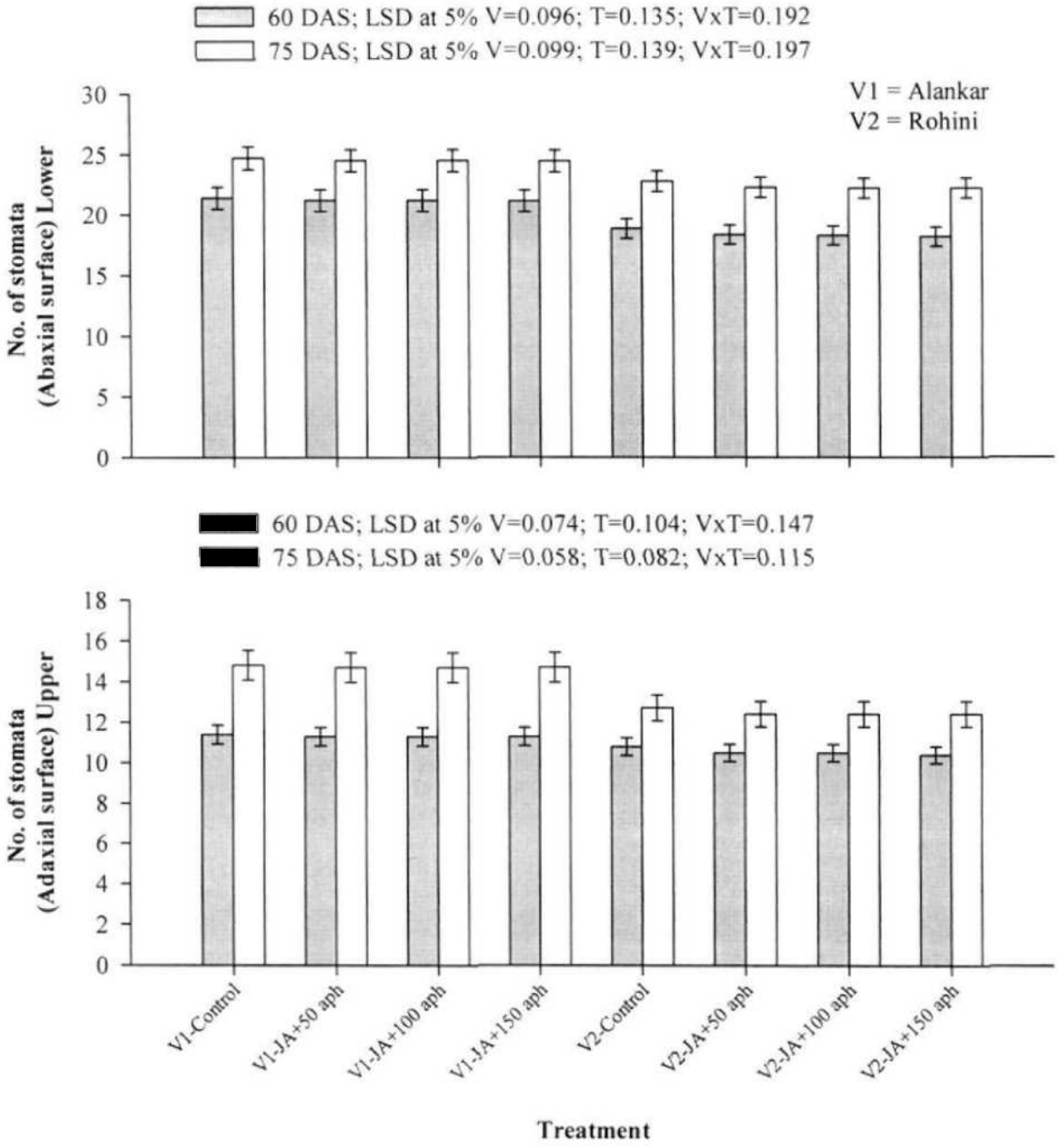


Fig. 119, 120. Combined effect of jasmonic acid (1.0 mM) and aphid infestation (50, 100 and 150 aphids per plant) on number of stomata on abaxial and adaxial leaf surface of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

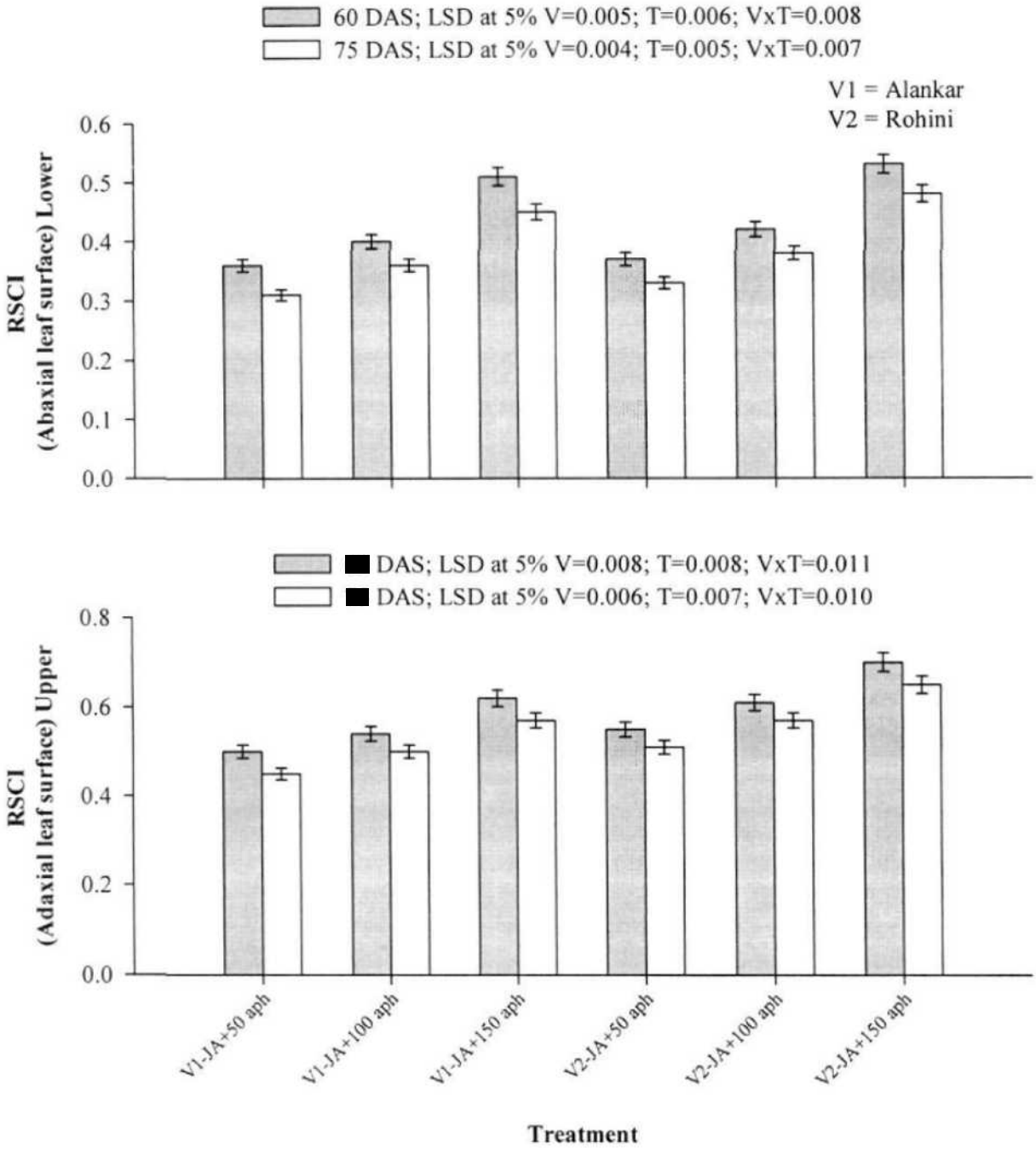


Fig. 121, 122. Combined effect of jasmonic acid (1.0 mM) and aphid infestation (50, 100 and 150 aphids per plant) on relative stomatal closure index (RSCI) of abaxial and adaxial surface of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

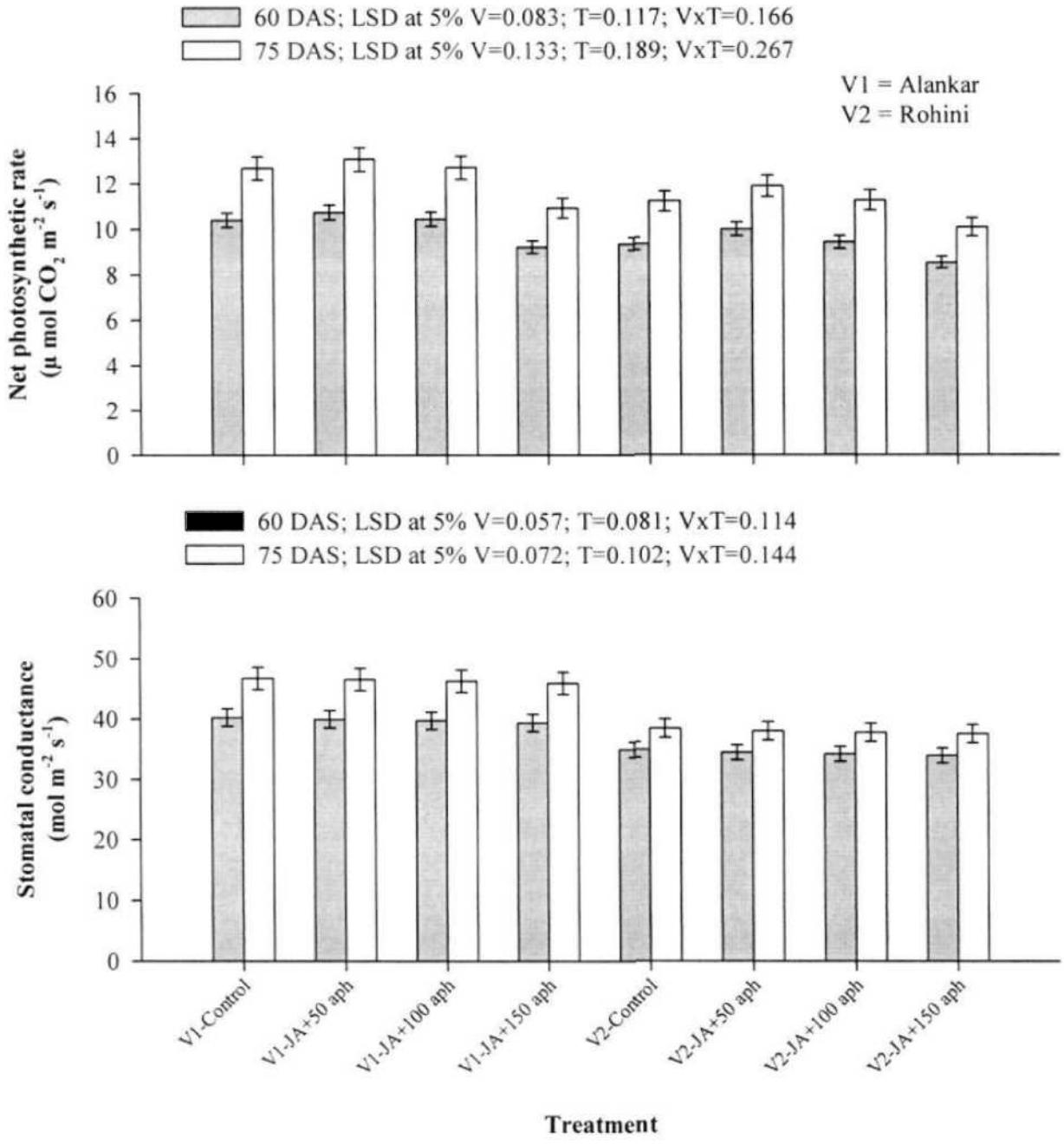


Fig.123, 124. Combined effect of jasmonic acid (1.0 mM) and aphid infestation (50, 100 and 150 aphids per plant) on net photosynthetic rate (P_N; μ mol CO_2 m^{-2} sec^{-1}) and stomatal conductance (g_s; mol m^{-2} sec^{-1}) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

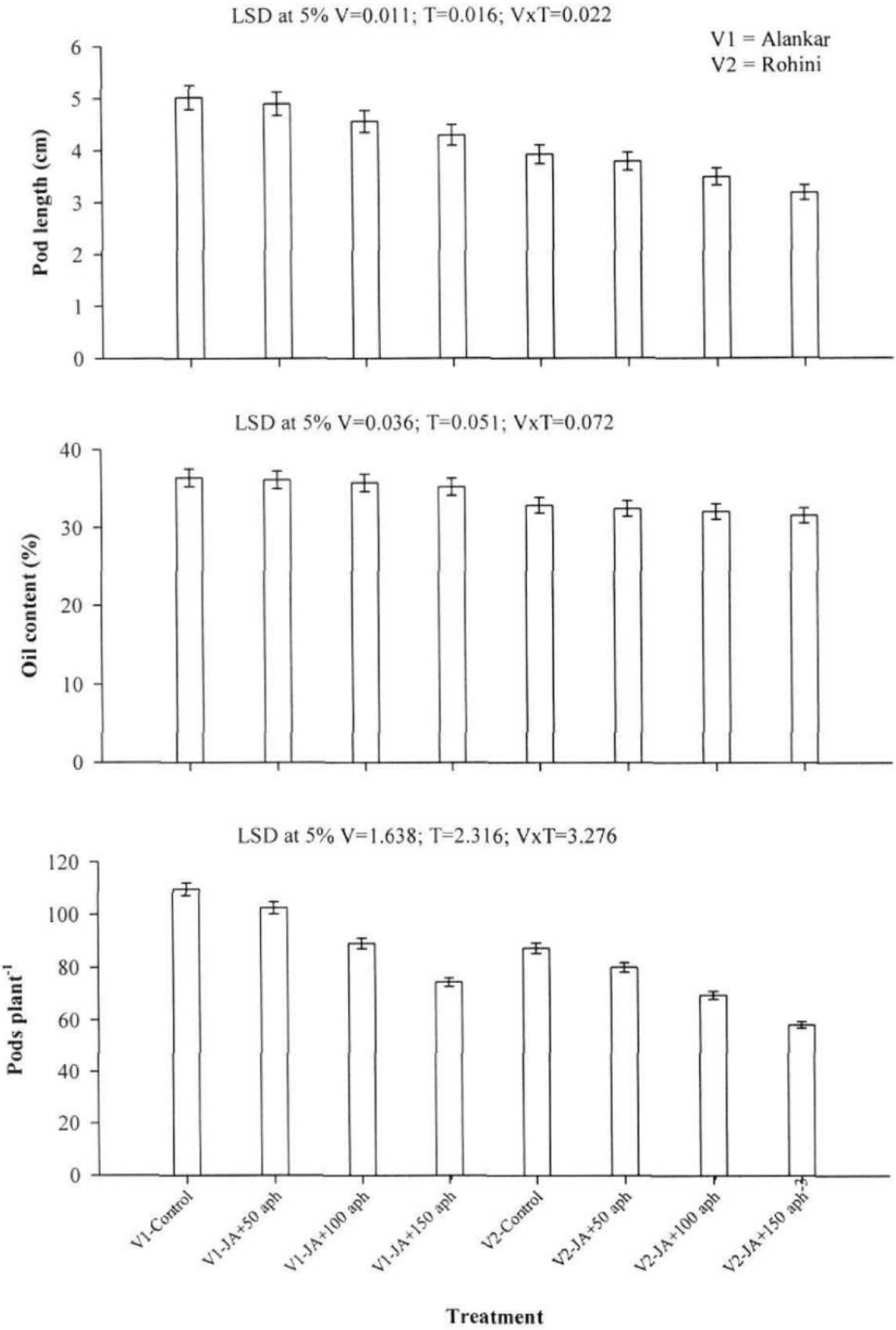


Fig. 125, 126, 127. *Combined effect of jasmonic acid (1.0 mM) and aphid infestation (50, 100 and 150 aphids per plant) on pod length (cm), oil content (%) and pod plant*$^{-1}$ *of Brassica juncea* cvs. Alankar and Rohini at harvest

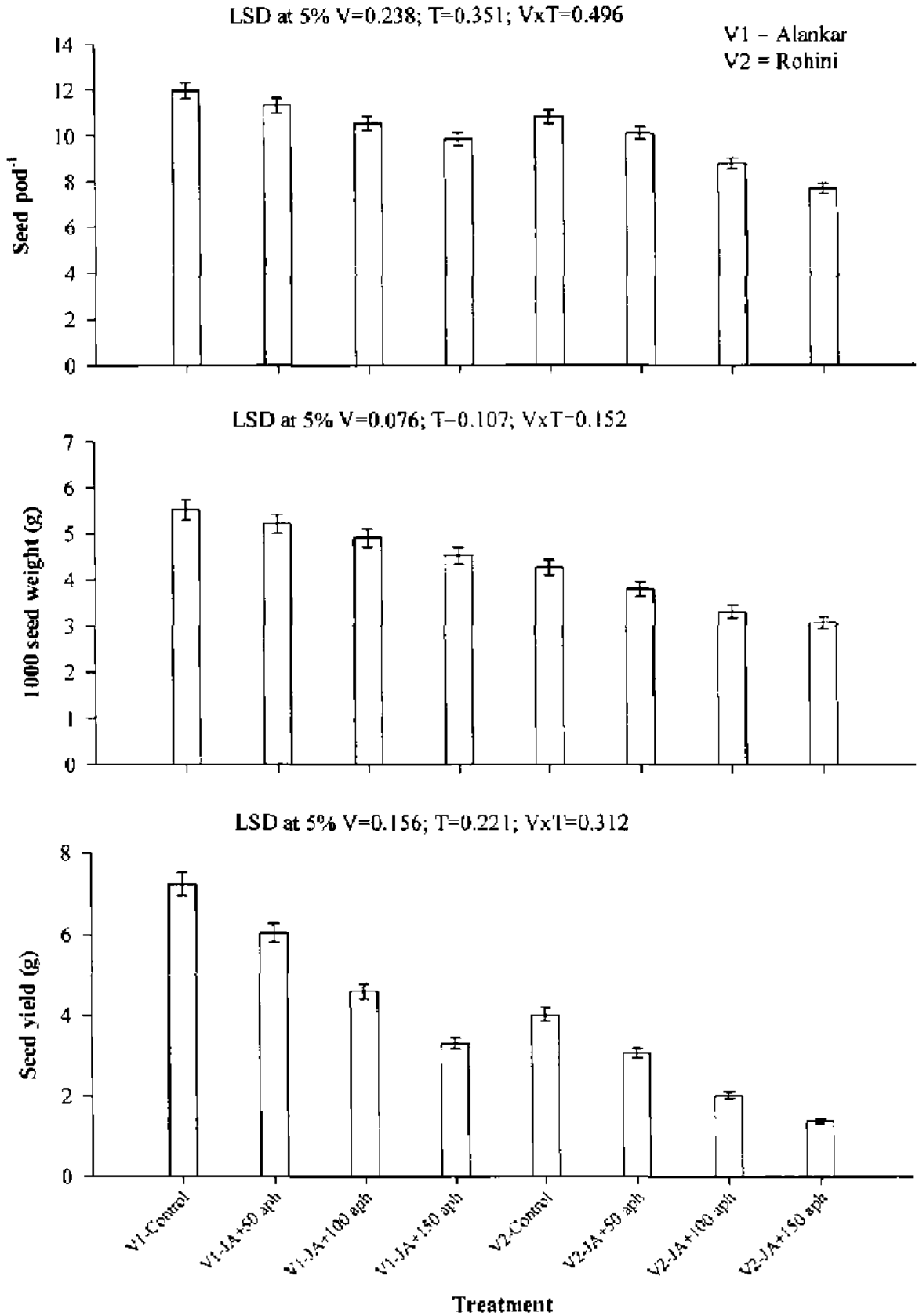


Fig. 128, 129, 130. Effect Combined effect of jasmonic acid (1.0 mM) and aphid infestation (50, 100 and 150 aphids per plant) on seed pod^{-1}, 1000 seed weight (g), seed yield (g) of *Brassica juncea* cvs. Alankar and Rohini at harvest.

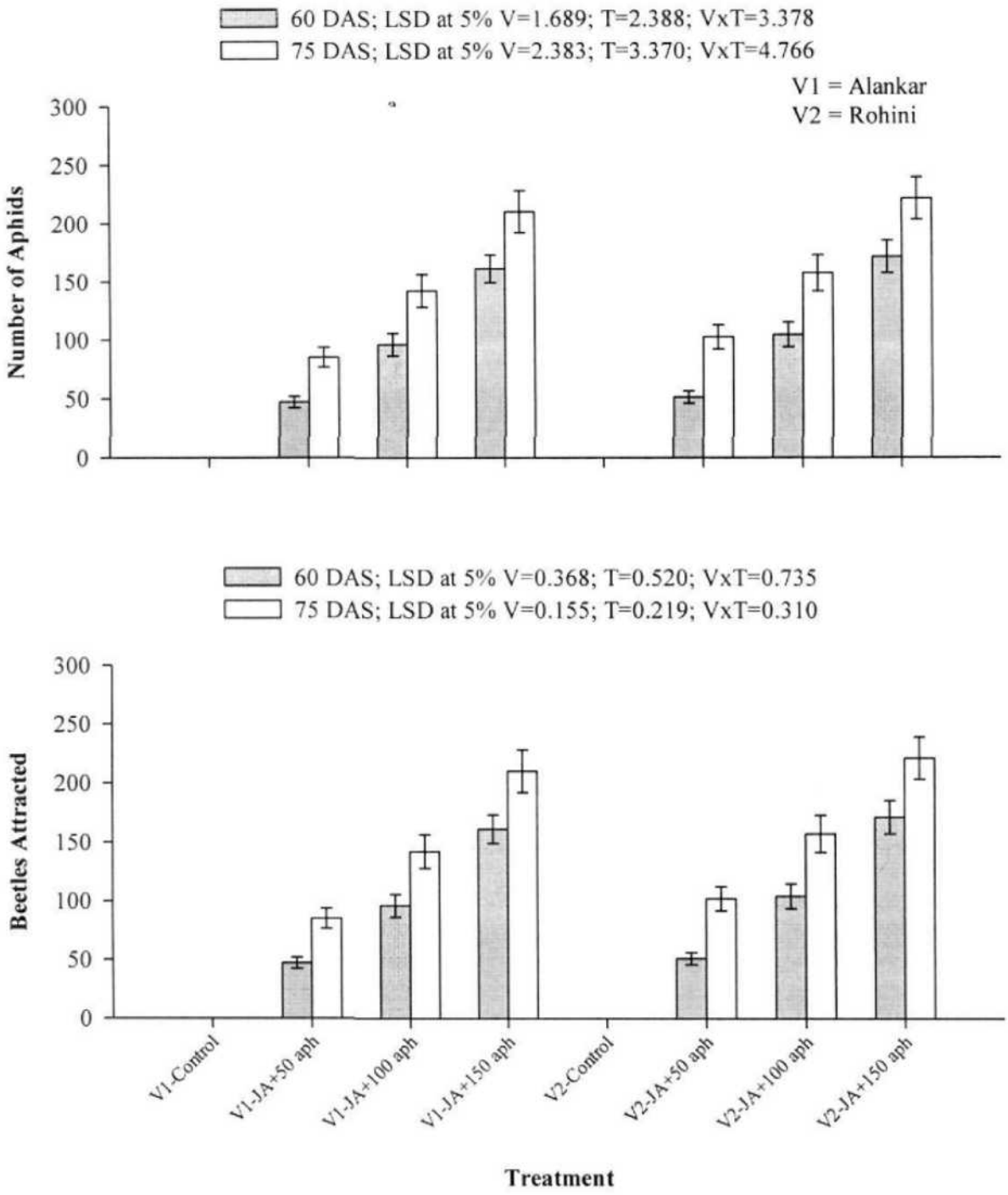


Fig. 131, 132. Population count of aphid and number of beetle attracted after combined effect of jasmonic acid (1.0 mM) and aphid infestation (50, 100 and 150 aphids per plant) on *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

S. No.	Treatments	Allyl-isothiocyanate content (%)	3-Hexan-1-ol content (%)
1.	Aphid(150)	42.36	10.34
2.	JA (1.0 mM) + aphid (150)	46.59	20.84

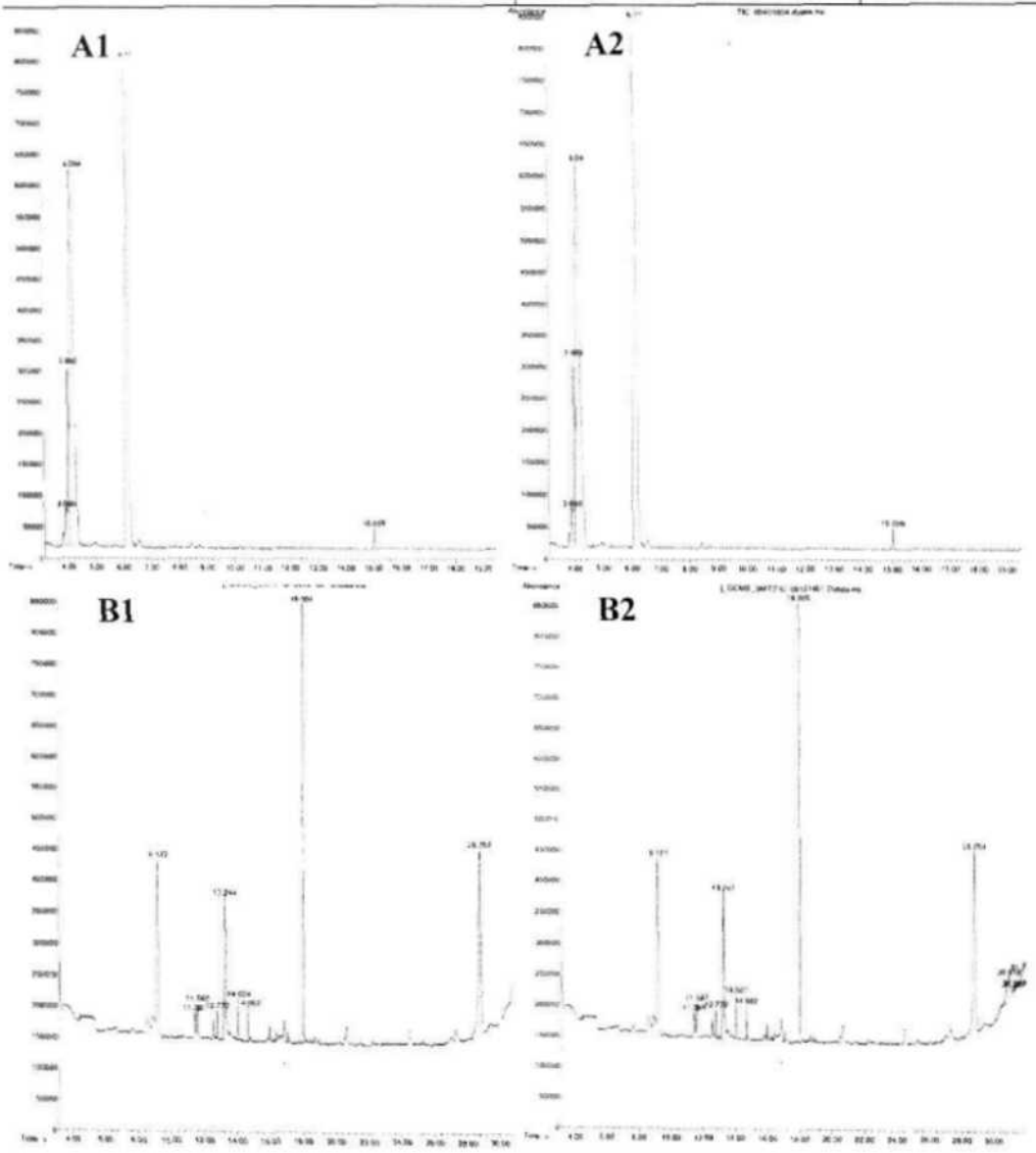


Fig. 133. Effect of aphid infestation (150 per plant) alone (A1, A2) or with pre-infestation spray of 1.0 mM jasmonic acid (B1, B2) on GS-MS profile of allyl-isothiocyanate and 3-hexan-1-ol content of *Brassica juncea* (cv. Alankar).

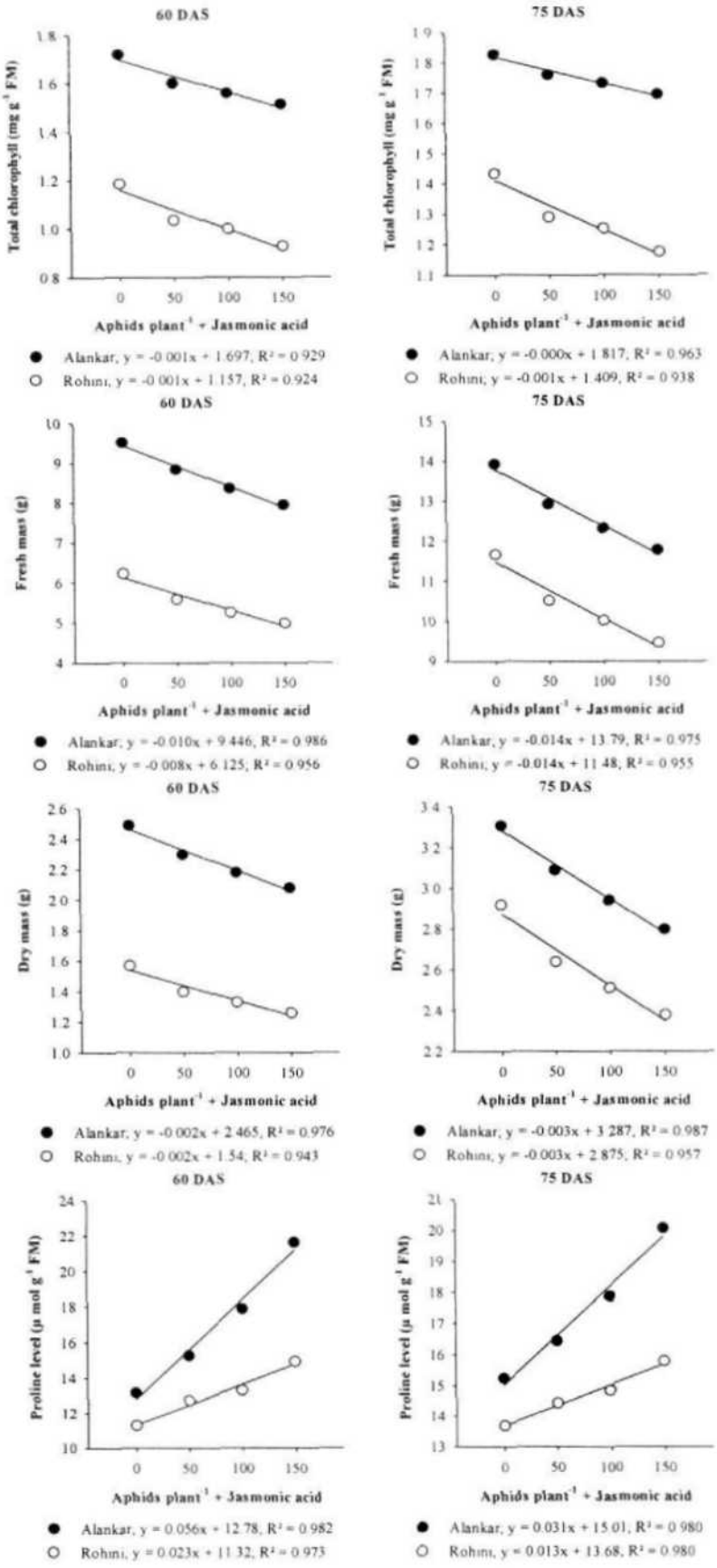


Fig.LR-IX. Linear Regression line with equation and squired correlation coefficient between various growth parameters vs. combined effect of jasmonic acid (1.0 mM) and varying aphid level (50, 100 and 150 aphid per plant) in selected cultivars at 60 and 75 DAS stages.

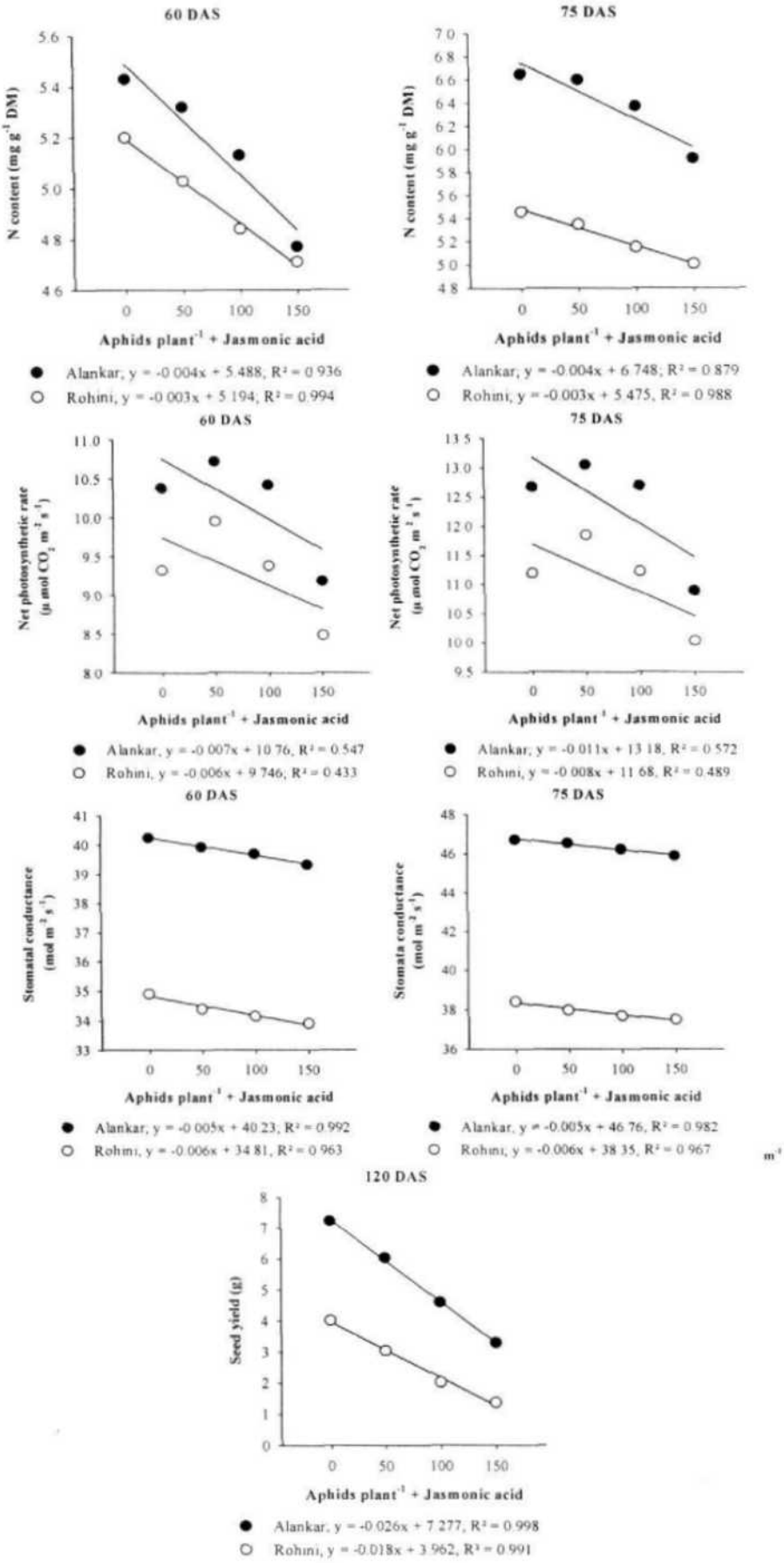


Fig.LR-X. Linear Regression line with equation and squired correlation coefficient between various biochemical and yield parameters vs. combine effect of Jasmonic acid (1.0 mM) and varying aphid level (50, 100, and 150 aphid per plant) in selected cultivars at 60 and 75 DAS stages

of 1.0 mM JA with 50, 100 and 150 aphids (Fig. 131). On treatment with 1.0 mM JA and 50 aphids, the aphid count was relatively low at 60 DAS stage in cultivar Alankar. But, the aphid population in cultivar Rohini increased in response to combined effect of JA + varying levels of aphid (Fig. 131).

A set of these plants were exposed to attract beetles and their numbers were counted. The cv. Alankar attracted the beetles more actively than cv. Rohini. The beetles count was higher at 60 DAS and their number eventually decreased at 75 DAS (Fig. 132, Plate 6).

Correlation coefficients and regression analysis

The correlation coefficients between selected parameters and 1.0 mM JA + aphid treatment were determined and linear regression lines plotted (Fig. LR- IX and X). The relationship of selected depended growth variables with independent variable (treatment with varying level of aphid +1.0 mM of JA) were linear, strong and negative, except in case of proline vs treatment, showing linear, strong and positive correlation (Fig. LR-IX and X).

Estimation of defensive volatile chemicals in aphid injured plants

The range of various signaling and volatile defensive chemicals sequestered in aphid injured plants were determined by GC-MS analysis of highly injured floral axis and leaves and compared with chemical sequestering in healthy un-injured leaves. The aphid injured leaves released a wide range of aphid repelling (deterrents) and toxic volatile chemicals in addition to beetle inviting volatiles. These volatiles were sequestered in aphid injured leaves and not by any component of floral axis. The higher quantities of allyl iso-thiocynates (AITC) and 3-hexanal-1-ol were detected in leaves pre-treated with JA and later injured by aphid (150) than in aphid injured leaves without prior treatment of JA (Fig. 133). The JA is known to induce stomatal closure besides direct defensive impact against aphid herbivory.

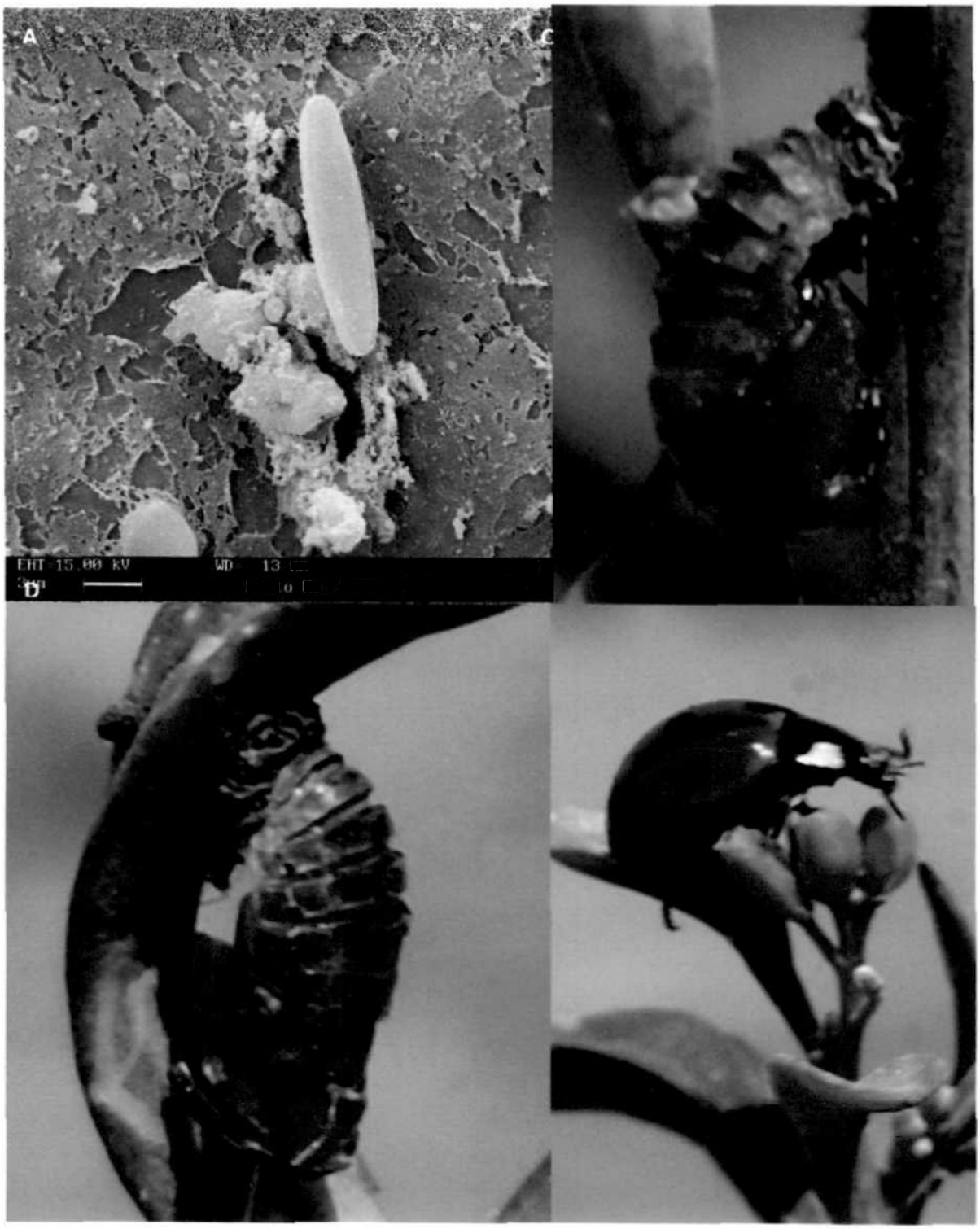


Plate 6. Few growth stages of ladybird beetle (*Coccinella septempunctata*) maintained on *B. juncea* cv. Alankar pretreated with jasmonic acid (1.0 mM) along with 150 aphids. A. SEM image of egg. B. Moulting . C. Moulted skin of beetle. D. Young beetle.

Chapter 5

Discussion

DISCUSSION

Ecosystems naturally consist of 3-5 trophic levels with chlorophyll bearing green plants at base constituting 1st trophic level. The herbivores consume plant parts and rebuild animal proteins form 2nd trophic level. The carnivores constitute 3rd to 5th trophic levels. In all ecosystems, the partitioning of energy from 1st to highest trophic level is naturally balanced. The highly evolved process of checks and balances in the grazing food-chain maintains a very delicate balance between the organisms at each trophic level and thus make the entire ecosystem self sufficient. An understanding of the key processes of checks and balances in energy (food) transfer may be of immense importance and use in agriculture and several other human welfares including ecology and environment. The aim of the present study was to understand various defense traits evolved and inherited by the plants and extent of counter offensive mechanisms in herbivores and their carnivores. In the present study, the defensive traits evolved and inherited by plant, their impacts on herbivory and predation; effects of herbivory on plant growth and population growth of herbivore and its predators have been studied. For this purpose, linear food-chain model of mustard-aphid-beetle (producer-herbivore-predator) was studied through five experiments.

The first experiment was conducted to study the relative sensitivity of five cultivars of mustard to herbivory by 40 aphids. The selected cultivars were grown in pots and at 45 days after sowing (DAS), each replicate plant was exposed to 40 aphids (*Lipaphis erisimi*). The growth responses of all the five cultivars were studied and one least and one most sensitive cultivars among the five were screened. The plants are directly exposed to environmental stresses including biotic stress caused by herbivores. The host plants have evolved several defense strategies including production and release of certain volatile chemicals with deterring or phytotoxic effects against aphids and to inviting signal the predatory beetles. All these traits are not equally evolved in plants. The types and levels of constitutive and induced defenses differ species to species and cultivar to cultivar and accordingly plants differ in growth responses to herbivory as well. Before proceeding for detailed studies on defense signaling and responses to herbivory, it was deemed fit to determine the least susceptible and most susceptible cultivar of *Brassica juncea* (referred as mustard) to

aphids (*Lipaphis erysimi*). It emerged from this experiment, that cultivar Alankar was relatively least susceptible to aphid herbivory and feasibly had better inherited traits of defenses than most sensitive cultivar Rohini.

Aphids are considered successful herbivore with soft bodies, membranous wings and a diet comprised of phloem sap (Dixon, 1998). In the present study, growth of plant (plant length, leaf area, fresh plant mass, dry plant mass, chlorophyll content and protein content) decreased in proportion to the increase in aphid population in all the five selected cultivars (Fig.2-12, and 14). Defense proteins increase resistance in plants (Elzinga and Jander, 2013). The protein contents were higher in cv. Alankar than in cv. Rohini. The growth attributes had a high degree of correlation with the aphid population (Fig. LR I and II). Aphids are phloem suckers and their water as well as nutrient requirements are fulfilled through the consumed phloem sap. The direct intake of phloem sap increase osmotic potential in aphids' gut and to compensate it, aphid sucks water from the xylem tissues (Spiller et al., 1990; Walling, 2008). The cultivars with high population densities of aphids partitioned proportionately high quantities of phloem sap (water and photosynthates) to herbivorous aphids as is evident from correlation studies (Fig. LR I and II). On aphid attack, the selected cultivars suffered from abundant availability of photosynthates for cell division and adequate water for the turgor pressure required during cell expansion. The correlation coefficients and linear regression between per cent reduction in fresh plant mass (Fig. LR I and II) and per cent increase in aphid population (Fig.1) explicitly explains the loss of water from plant tissues was due to consumption by aphids and thereby caused stress in plant tissues (Khattab, 2007; Sadek et al., 2013). It is also evident from the excess accumulation of proline that plant has suffered from water stress. Therefore, proline besides playing important role in plant defenses, also managed the osmotic imbalance in mustard (Oncel et al., 1996). The correlation coefficients between plant dry mass (Fig. LR I and II) and increase in aphid population established that growing aphid population consumed excessive quantity of photosynthates and this loss of carbon may have suppressed cell division in root and shoot. Walling (2000) reported that phloem sap herbivory adversely affected the plant productivity (Bak et al., 2013; Sadek et al., 2013).

The presumption that phloem sap directly consumed by aphids caused water stress in the plant tissues is further supported from high degrees of correlation

between proline content and aphid population in varying cultivars (LR I and II). The proline in water stressed conditions acts as water stress adjuster in plants (Khattab, 2007). The photosynthetic pigments, leaves and plant mass in both selected cultivars were more severely affected than roots. It may be due to severe cell damage caused by aphid in above ground leaves, stem and inflorescence. The minute loss to roots may have been due altered carbon partitioning and loss of carbon in above ground plant parts. Aphid infestation reduced photosynthetic pigments level (Fig. 9-12), proteins (Fig. 14) and other growth attributes (Fig. 2-8) in all the cultivars of *Brassica juncea* (El-Khawas and El-Khawas, 2008). This finding is in agreement with the earlier report that insect infestations in wheat crop inhibited chlorophyll biosynthesis (Heng-Moss et al., 2003).

Usually, dose-response variables are in common practice of correlation studies. The responses of one species to varying doses of aphids establish relationship at individual species level only. The cultivars of any single species differ in the inherited traits for resistance, defensive strategies, growth and yield potentialities. The treatment of five cultivars with 40 aphids per plant and correlation of cultivar-response variables establish a community level response.

In this experiment, growth responses of the two selected mustard cultivars (Alankar and Rohini) to varying quantities of aphids (50, 100, and 150 aphid per plant) were studied. The reduction in shoot and root length, leaf number and area of both the cultivars increased with the number of aphids (Fig. 15, 16, 17, 18). The reduction in growth parameters was higher in cv. Rohini than in cv. Alankar. It is reported that, aphid devitalized the crop by sucking the cell sap (Bakhetia 1991, Atri et al. 2012). In the present study, the fresh mass reduced at every aphid infestation level. The reduction in fresh and dry mass was higher on infestation with 150 aphids per plant (Fig. 19, 20). It is evident from the Fig. 43 that the population of aphid multiplied more quickly on Rohini than in Alankar. The aphid count corresponded with the initial number of aphids inoculated. The aphids are phloem sucker and directly consumed photosynthates. The concentration of carbohydrates in the phloem sap create an osmotic imbalance in the aphid gut (Walling, 2008). To avoid dehydration, aphids maintain water balance by occasional feeding through the xylem (Spiller et al., 1990). It is evident from the finding on fresh mass and dry mass of both the cultivars at selected aphid infestation levels. The fresh mass in the plant is due to

the water content and dry mass is the water organic matter. The reduction in both fresh and dry mass indicates the loss of carbohydrate and water from the tissues in proportion to aphid numbers. The higher degree of weight loss in Rohini than in Alankar appears to be due to the difference in inherited defense traits (Fig. 19, 20). The proline level increased in both the cultivars in proportion to the number of aphid inoculated (Fig. 27).

The proline is a universal stress marker in plants (Oncel, 1996) and hence increased with the infestation level. Similar increase in free proline has been reported by (Khattab, 2005, 2007). In cabbage and eucalyptus leaves infested with aphid and therefore the proline accumulation was considered as biotic stress marker. The proline accumulation is speculated to play a defensive role (Kuznetsov and Shevyakova, 1997). The greater amount of proline in cv. Alankar than in cv. Rohini indicates that the former cultivars had some inheritance resistant traits than the later cultivars.

The nitrogen (N) and phosphorus (P) content also decreased in proportion to the number of aphid inoculated (Fig. 28, 29). The insect feeding alters translocation pattern and growth of host plant (Miles, 1999) and reduced the nutrients uptake form the root (Wu et al., 2004). In the present study the N content decreased significantly with increase infestation level in both the cultivars (Fig. 28). It is reported that N enhance the reproductive capacity and resistance against insect damage (Forno and Semple, 1987). From the linear regression analysis it is evident that N content had strong negative relationship with the aphid infestation level in both cultivars (Fig. LR III and IV).

The reduction in nutrient contents due to aphid infestation many have directly affected the chlorophyll a, b, total chlorophyll and carotenoid content (Fig. 21-24). The loss of chlorophyll may have been caused by consumption of nutrients by the aphid, as pigment biosynthesis is dependent upon water and minerals. The aphid infestation reduced stomatal number in both the cultivars (Fig. 31, 32). The relative stomata closure index (RSCI) increased with the aphid infestation level but more prominently in cultivar Rohini (Fig. 33, 34).

In the present study, aphids caused severe damages of wax layer deposited on the outer surface of leaf epidermal cuticle (Plate 7). The cell membrane around the area of injury is also affected adversely. The loss of membrane permeability results

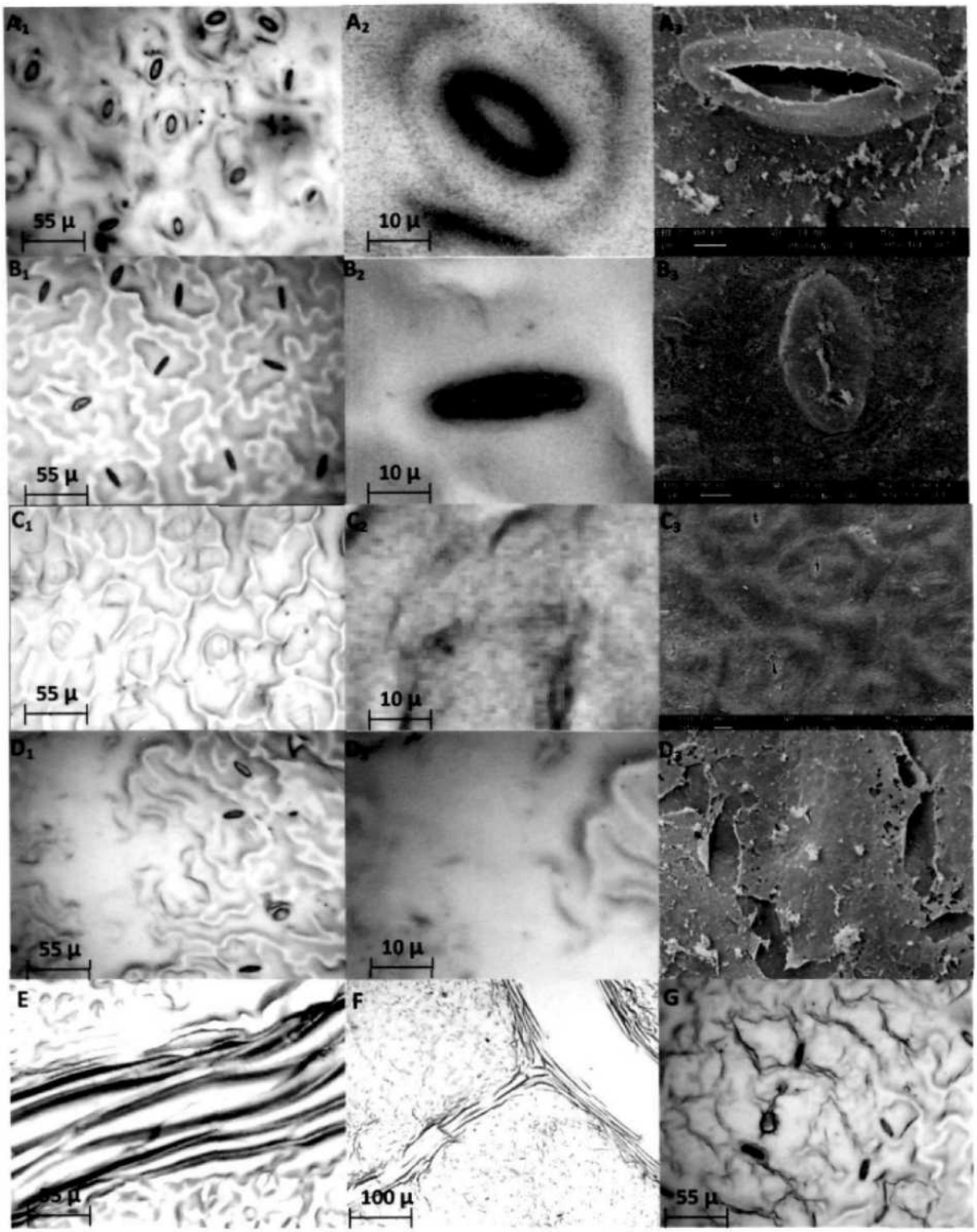


Plate 7. Features of leaf surface with stomata under 10×10x light microscope (A1-G). Details of leaf surface scanning electron microscopic (SEM) image (A3, B3, C3, D3). (A1-A3) open stomata in non-infested leaves of Alankar (control), (B1-B3) partially closed stomata without much deformation of wax layer on herbivory with 150 aphids per plant, (C1-C3) the damaged stomata wax layer in cv. Rohini infected with 150 aphids, (D1-D3) highly damaged wax layer and almost deformed and dissolved stomata in cv. Rohini infected with 150 aphids, (E1) Relatively intact sieve tube cells in the leaf veins of cv. Alankar treated with 150 aphid, (F) damaged sieve tube elements in the leaf vein of cv. Rohini treated with 150 aphids, (G) highly damaged guard cells and wax layer in cv. Rohini infected with 150 aphids

into the spill of cell water and vital contents (Walling, 2000; Louis and Shah, 2013). The hypertrophy noted in the mustard pods (Plate 3, 7) may have been caused severe cell damages in developing pods. Despite partial or complete closure of stomata, the fresh mass of plant also decreased on various level of aphid infestation (Fig. 19). This indicate that the reduction in fresh mass of plant was due to the consumption of water from the xylem in addition to photosynthates as evident from loss of plant dry mass as well (Fig. 20). The partially closed stomata as noted in aphid infested mustard plants (Plate 5) may have not checked transpiration completely, instead reduced the stomata conductance (Fig. 36) and hence affected the photosynthetic rate (Fig. 35). The reduction in pod length, oil content as well as pod per plant and seed weight in proportion to the aphid infestation level appears to be the outcome of reduction in photosynthetic rate and consumption of phloem sap by the aphid in proportion to their population (Fig. 37-42).

From the experiment, it is inferred that both the selected cultivars had difference in their inherited defensive traits. The increase in the degree of loss in various growth parameters increased with the infestation level and growth stage in both the cultivars and had a direct relationship with the aphid numbers. The relationship between the loss of selected growth parameters and aphid number is evident from linear regression analysis (Fig. LR III and IV). There was a strong correlation between the losses of net photosynthesis with respect to aphid infestation level. The strong and positive correlation coefficients were recorded between proline level and aphid infestation level in both the cultivars (Fig. LR III and IV).

In the third experiment, the selected cultivars (Alankar and Rohini) of *Brassica juncea* were exposed to varying number of aphid with equal degree of predation (2 beetles per plant). Almost all the selected parameters showed reductions in proportion to number of aphids inoculated. The cultivar Rohini remained more susceptible than cultivar Alankar despite the predation of aphids by beetles. The decrease in fresh and dry mass caused by aphid infestation adversely affected the plant development in terms of shoot and root length, leaf number and area, (Fig. 45-48). The loss in dry mass corresponding to number of aphid feeding on the plant indicated that larger number of aphid neutralized the defensive responses of host plants and limited population of beetles may have not been able to predate excessively on multiplying aphids. The aphids, besides gelling saliva also secrete watery saliva

after piercing stylet into the plant tissues (Bak et al., 2013). The watery saliva plays important role in testing the suitability of the cell sap of the host plant (Bak et al., 2013). Recent analysis of saliva protein reflected that some of the proteins known as elicitors trigger the plant defense responses while some other counteract the plant defense as affector molecules. Some of these molecules might be species specific and some other have broader specificity (Bos et al., 2010; Tian et al., 2012; Pitino and Hogenhout, 2013; Bak et al., 2013). It is likely that cultivar level variations in such inherited defensive traits may have caused varying levels of damages in the two selected cultivars. In the present study the degree of losses in the various growth and biochemical parameters were lesser on infestation with 50 aphids (Fig. 49-66). This indicate that the selected cultivars of mustard had limited constitutive and induced defensive mechanisms against aphids in the presence of only two predatory beetles (*Coccinella septempunctata*). The higher number of aphids could damage the plant to a greater extent. It is also reported that the plant degradation products are recognized by plant receptors during aphid herbivory; however, certain effectors from aphid saliva neutralize plant defense responses to re-established plant susceptibility (Liu et al., 2009; Pitino and Hogenhout, 2013; Bak et al., 2013). This immunity to plant defense caused by aphid saliva proteins acting as effectors in the larger number of attacking aphids enabling them to successfully consume greater amounts of carbon to substantially reduce plant growth at higher level of aphid infestation (Jones and Dangl , 2006; Liu et al., 2009; Bak et al., 2013). Despite equal level of predation the cultivar Rohini remained more susceptible to aphid infestation than Alankar. It is reported that Orthopod feeding change plant metabolism and gene expression associated with plant defense responses and aphid resistance gene (Moran and Thompson, 2001). It is evident from the data on aphid count on both the cultivars that population of aphid consistently increased with age of the plant (Fig. 73). The large aphid population on cultivar Rohini than cultivar Alankar may have been due to gene level variations between them.

It may be noted that the limited number of beetles (2 beetles per plant) could reduced the aphid population to a limited extent as compare to the infestation by aphid without beetles in Experiment 2 and eventually the growth and yield of selected cultivars (Fig 49-72). On comparison of results of the Experiment 2 and 3, the growth of both cultivars was relatively better under predation by beetles (Experiment 3) than

without predation (Experiment 2). The beetles also reduced the aphid population in Experiment-3, but insufficient number of beetles and possible reversal of plant immune and defensive system by larger number of aphids reduced the growth of mustard in Experiment 3.

The selected cultivars (Alankar and Rohini were treated exogenously) with varying doses of jamonic acid (JA) to study the effect on the growth, physiology and yield as well as extent of inviting signal to beetles. The JA application of 0.1 mM enhanced the growth and yield of both the cultivars (Fig. 74-101). Jasmonic acid is a key molecule of octadecanoid signaling pathway (Meyer et al., 1984, 2003, Markunas et al., 2011, Nabity et al., 2013) which strongly stimulates photosynthetic pigment synthesis (Poonam et al., 2013). It also protects the cell membrane in accordance with the dose of JA (Poonam et al., 2013). Jasmonate treatment reduces the proline level in the plants as compare to the aphid attacked ones (with or without predation). This indicates that JA itself reduced plant stress, thereby; proline did not accumulated in larger proportions in JA treated plants. Since proline is considered as a stress marker (Oncel, 1996), reduced proline accumulation indicated that JA treatment stimulated and protected the cellular stress and relatively healthy cells in selected cultivars defended the plants better. In earlier studies JA treated stressed and non stressed plant had mixed response on accumulation of proline (Poonam et al., 2013, Jamalomidi et al., 2013). The increased chlorophyll content in JA treated plants, increased plant growth specifically the fresh and dry mass (Fig. 80-95). The JA treated cultivars Alankar and Rohini attracted larger number of beetles in the present experiment at 60 DAS than at 75 DAS (Fig. 102). This indicates that 1.0 mM of JA signaled beetles very effectively only at 60 DAS and relatively to a lesser extent at 75 DAS as effect of this volatile chemical reduced with time. The JA is reported to play on important role in aphid resistance as well (Smith and Boyko, 2007, Morkunas et al., 2011). The JA treatment enhanced the plant's immunity to aphid attack. It may be noted that JA application reduced the aphid reproduction in an earlier study (Zhu- Salzman et al., 2004).

In the fifth experiment, both the cultivars (Alakar and Rohini) pretreated with 0.1mM Jasmonic acid (JA) at 45 DAS were inoculated with 50, 100, and 150 aphid per plant at 50 DAS, and the growth performance was studied. On pre-treatment with JA, the plant growth enhanced far better than without JA treatment (Experiment 2).

The inoculations of aphids on JA pre-treated cultivars reduced shoot and root length to a lesser extent than in Experiment 2 (Fig. 103-104). The population of aphid on JA pre-treated cultivars was also lesser than without JA treated plant but exposed to beetles as recorded in Experiment-3. The population of aphid on pre-treated (JA 0.1mM) plants was relatively lesser than in plants without JA. From this experiment (Fig. 129), it is evident that exogenous application of 1.0 mM of JA detracted the aphids due to adverse impact on their reproductive abilities (Zhu- Salzman et al., 2004) and substantial attraction of beetles had added advantages to safely predate on them before the aphids could introduce defensive chemicals in them. The JA also provided a cue to predators more effectively in presence of aphid.

The defensive volatiles synthesized and released by leaves of cultivar Alankar inoculated with 150 aphids per plant was studied in GC-MS analysis. The profile reflected that the content of total allyl-isothiocynate (AITC) was only 42.36% and 3-hexan-1-ol was 10.34%. When this cultivar was treated with 1.0 mM of JA prior to inoculation of 150 aphids, the contents of both these defensive and signaling volatiles increased. The AITC increased up to 46.59% and 3-hexan-1-ol up to 20.84% (Fig. 133). These findings firmly suggest that the exogenous application of JA on mustard plants improved plant's ability to defend the aphid herbivory as AITC directly deter the aphids and JA spray worked as volatile signal to attract predatory beetles. The induced defenses in many plant species through volatile chemical arsenals have been reported earlier also (Lambrix et al., 2001; Kliebenstein et al., 2002; Pontoppidan et al., 2005; Kissen et al., 2009).

The overall outcome of the present studies (Experiment 1-5) has been explained through the Plate 8. The aphid attack is more common on mustard cultivars with least inherited defensive traits. The plants have evolved constitutive and induced defensive traits against herbivores. The herbivores in turn have co-evolved counter defenses against the defensive plant volatiles and thus developed the ability to alter certain blends of plant volatiles in their favor. Herbivores often alter the plant volatile signaling specialist predators or parasites of the attacking herbivore (Bak et al., 2013). In the present study it is evident that the selected aphid damaged the wax layer and feasibly secreted enzyme pectinase, dissolved the middle lamella between epidermal cells and subsequently the middle lamella of cells/tissues in leaf interior (mesophylls, cortex in stem, phloem cells, primary xylem cells in the vascular system of leaf and

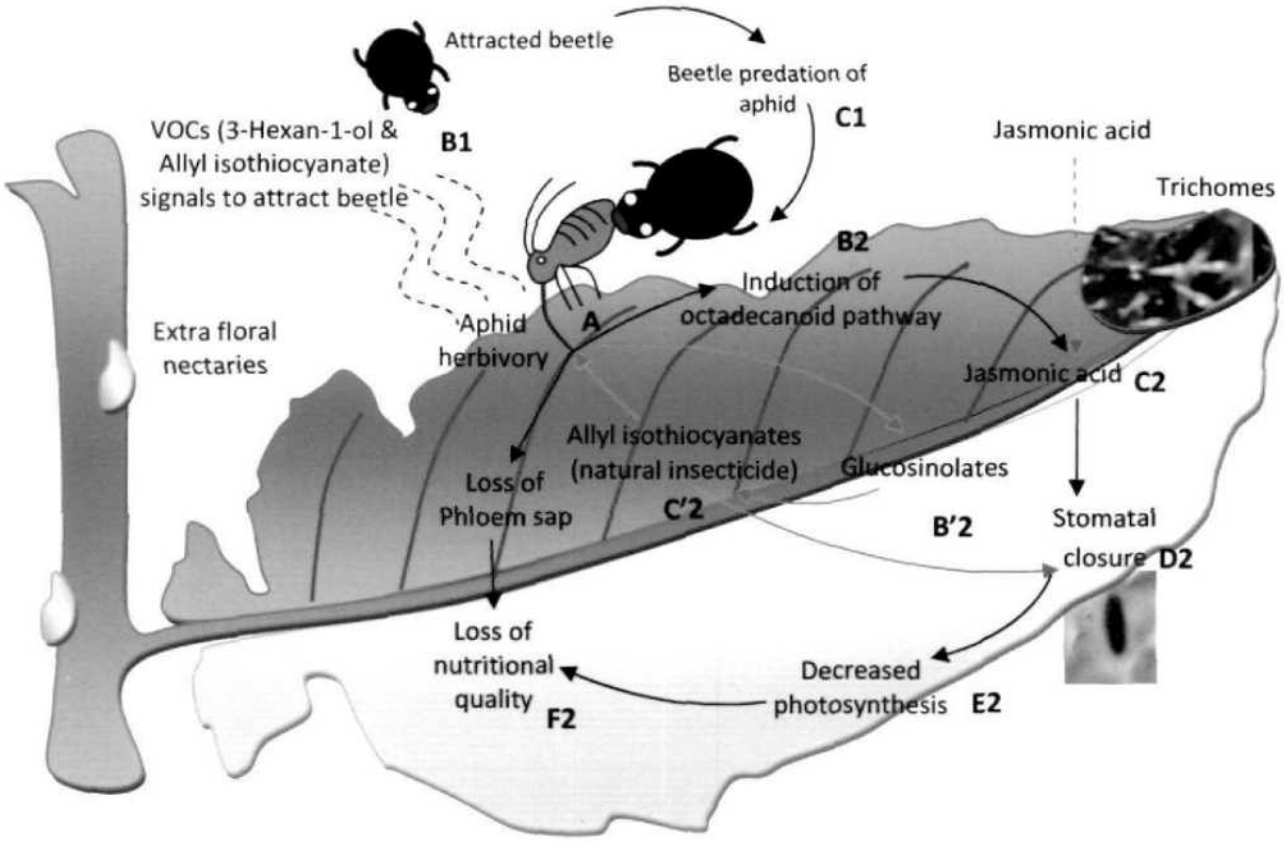


Plate 8. Tri-trophic interaction of *Brassica juncea – Lipaphis erysimi - Coccinella septempunctata* (mustard plant – aphid – beetle). Attack of aphid on mustard plant results into loss of phloem and low of nutritional quality. However, this (A) led to release of volatile signals which attracts beetles (B1) which feeds on aphid to check their population (C1). Aphid feeding also induces octadecanoid pathway to release jasmonic acid (B2). Elevated level of jasmonic acid closes stomata (D2) to decrease photosynthesis (E2) further reduces nutritional quality (F2). Alternatively, pre-infestation external application of jasmonic acid (blue arrows) induces glucosinolates (B'2) which on infection quickly releases allyl isothiocyanates to deter aphids (C'2).

stem). The aphids thus easily enter their stylets through the macerated cells and immediately secrete the gelling saliva and fill in the injured tissues around stylet probing sites. The gelling saliva secures the stylets. The purple brown pigmenting substance filled in the injured portions of leaf and stem has been detected in the present study and shown in the plates (Plate 3). The probing aphid first tastes the contents of the cell sap by injecting watery saliva inside the cell and withdrawing the cell sap. On judging the suitability of cell sap, aphids enter the probing stylet into sieve tube and suck in the photosynthates synthesized in the leaves and unloaded in the phloem. The excessive sugar intake increases osmotic potential in the aphid guts. To nullify this effect, aphids enter their stylets into the xylem cell and withdraw water. The mustard cultivars with better defensive traits (a set of few genes) activate the sequestering several blends of volatiles. Some volatiles deterred the aphids and some other signalled the predatory beetles of third trophic levels for indirect control. The cycles of deterring and predator cueing volatiles are shown in the Plate 8. Certain blends of volatiles are known to signal the specialist predator about the suitability of egg laying sites. In the present study, the specialist beetle (*Coccinella septempunctata*) preferred to maintain its life cycle on cultivar Alankar pre-treated with 1.0 Mm of JA (Plate-6).

Conclusion

Present study draws following conclusions to answer the objectives framed (see Chapter 1) for the conducted experiments

1. The selected mustard cultivars (Alankar, Pusa Jai Kisan, Varuna, Sakha and Rohini) responded differently against selected aphid infestation (40 aphids per plant) at the two stages of growth. Alankar stood least sensitive against aphid herbivory amongst all the five mustard cultivars whereas the response of Rohini was most susceptible.
2. The number of aphids increased linearly once their selected numbers were inoculated on young leaves of plant, which caused increasing damage with the growth progression (60 to 75 DAS).
3. The herbivory damage increased from 60 to 75 DAS which was more pronounced in cv. Rohini as compared to cv. Alankar. Moreover, stress

progressively increased with increasing the level of aphid infestation (0-150 aphids).

4. Inoculation of predatory beetle controlled the aphid population which was observed as recovery of selected parameters as compared to aphid infestation alone. The damage declined with the age progression (60 to 75 DAS).

5. Amongst the three concentration of jasmonic acid (0.5, 1.0 or 1.5mM) the response of 1.0 mM was most effective which furnished positive responses of selected parameters at the two stages of growth.

6. Pre-treatment (aphid pre-infestation spray) with jasmonic acid with JA solution effectively controlled the aphid infestation which resulted in better growth performance of cultivars as compared to aphid stressed plants, more at late stage of growth (75 DAS) than at early stage (60 DAS). Also the yield improved with the simulated jasmonic acid application.

7. Marked increase of proline in Alankar (least sensitive cv.) as compared to Rohini (most susceptible cv.) suggested its key role in protecting plants against herbivory induced damage.

8. Jasmonic acid (1.0 mM) application induced the proline level further confirms its protective role in herbivory induced plant growth regression.

9. Detection of higher level of allyl isothiocyanate (AITC) and 3-hexanal-1-ol in plants treated with JA pre-infestation to aphids as compared to aphid infestation alone confirms the applicability of JA against herbivores through the induction of direct and indirect defense.

Chapter 6

Summary

The objective of this study was to test the utility of herbivory simulation using jasmonic acid on some mustard cultivars. Considering this objective as framed in introduction of the thesis, five local cultivars were first screened for their relative sensitivity to aphid attacks. The problems of aphid infestation on crop plants, direct and indirect plant defenses responses, the role of jasmonic acid (JA) as simulator of natural herbivory to elicit the plant defense prior to aphid infestation have been emphasized in the *introduction* of the thesis. The relevant studies available with literature have been reviewed comprehensively in the following Chapter 2. The details of the biological materials used, methodologies used and preparation of chemicals reagents have been elaborated under the section *materials and method* with necessary details. The finding of the 5 experiments were statistically analyzed and presented in the graphical forms. The data tables have also been annexed at the end of the thesis. The last section deals with the explanations of the results extracted from the data and are discussed and interpreted in the light of the earliar relevant findings of other researchers in the field. A brief account of these chapters is summarized below:

Five pot experiments were conducted in winter season (October to March) of the year 2009-2011 at the Aligarh Muslim University, Aligarh. The objective was to elucidate the simulatory priming effect of JA and aphid (*Lipaphis erysimi* Kalt) individually and as follow up treatment after screening of the five *Brassica juncea* cultivars viz. Alankar, Pusa Jai Kisan, Varuna, Sakha and Rohini, against selected population of aphid. The effect of predatory ladybird (*Coccinella septempunctata*) on plant responses and aphid population also studied in the following experiments.

Experiment 1

This experiment was worked out to assess the relative susceptibility of the five local cultivars of mustard namely, Alankar, Pusa Jai Kisan, Varuna, Sakha and Rohini. The experiment was set up in the ambient conditions of winter season of the year 2009 in Aligarh Muslim University, Aligarh. These mustard cultivars were exposed to aphid infestation at the rate of 40 aphids (*Lipaphis erysimi*) per plant at 45 days after sowing. The seeds of all selected cultivars were sown in earthen pots. The selected cultivars were infested with selected and identified aphids (*Lipaphis erysimi*)

at the rate of 40 adult aphids per plant and a control of each cultivar without aphid was also maintained. Each set of five cultivars were arranged in completely randomized block design in separate, especially designed net houses of size 185×100×125cm supported with iron rods and zipped entrance. The seeds were sown in earthen pots of 25 x 25 cm size filled with garden soil and compost in the ratio of 3:1. All the five cultivars were sampled at 60 and 75 DAS (days after sowing) to analyze the growth attributes of infested plants and also the population growth of aphid on each cultivar, the plant growth responses (length of shoot, root, total plant, leaf number and area and plant fresh and dry mass), pigment concentration (chlorophyll a, b, total chlorophyll and carotenoid), total protein and proline contents. All the growth attributes and biochemical parameters decreased significantly on aphid herbivory at both the sampling stages (60 and 75 DAS), whereas, only proline content increased on aphid infestation. The damage of all the cultivars increased as the population of aphid multiplied with plant age. On the basis of increasing sensitivity in terms of per cent loss, the selected cultivars can be arranged from least to most sensitive as Alankar < Pusa Jai Kisan > Varuna > Sakha > Rohini. Alankar, therefore, was relatively most resistant to aphid supporting lowest number of aphid population and least resultant damage, whereas, Rohini was screened as most susceptible cultivar with highest aphid population and damage. One set was kept in open environment to naturally invite the beetles on aphid infested plants and beetle number was counted. The cultivar Alankar attracted more beetles than Rohini.

Experiment 2

Two cultivars i.e. most susceptible to aphid infestation (Rohini) and least susceptible (Alankar) were screened out from Experiment 1. This experiment was set up in the growth season of the successive year keeping all the cultivation practices and statistical design same as in Experiment 1. Each of the selected variety (Alankar and Rohini) was independently infested with 0 (control), 50, 100 or 150 aphids per plant at 45 DAS. The comparative response of growth, biochemical, physiological and yield parameters were recorded and analyzed in detail at 60 and 75 DAS, and finally harvested at 120 DAS. Besides, reduction in growth parameters (length of shoot, root, total plant, leaf number, leaf area, fresh and dry plant mass), aphids also induced adverse changes in the stomata and their dynamics (relative stomatal closure index,

number of stomata) and hence affected the gaseous exchange, net photosynthetic rate, photosynthetic pigments (chlorophyll a, b, total chlorophyll and carotenoids content), proteins and nutritional quality (NPK level) of the two selected cultivars. The increase of proline content reflected the protective response of cultivars against aphid induced water stress in plants. The decline in all these parameters contributed to decreased yield attributes (pod length, pods per plant, seeds per pod, 1000 seed weight and seed yield) including seed oil content. This decrease was more pronounced in cv. Rohini as compared to cv. Alankar. Higher level of proline in Alankar suggested better protective mechanism in this cultivar as compared to Rohini.

Experiment 3

Attraction of predatory beetles to aphid infested plants to show ability to defend indirectly and inherited tri-trophic signaling mechanism. Two selected cultivars of mustard were infested with 50, 100, 150 aphids per plant at 45 days growth stage and 5 days later (50 DAS), two beetles (Ladybird; *Coccinella septempunctata*) per plant were introduced. All the experimental designs were same as in Experiment 1. The plant samples were collected at 60 and 75 DAS to record growth, physiological, and biochemical characteristics of the two cultivars of Alankar and Rohini and finally harvested at maturity (120 DAS) for yield parameters. The aphid herbivory with 50 and 100 aphids + 2 beetles per plant in together with two predatory beetles per plant, reduced plant growth (length, fresh and dry mass of shoot and root, area and number of leaves per plant), adversely changed stomatal dynamics (relative stomatal closure index, number of stomata), photosynthetic performance (net photosynthetic rate, stomatal gaseous exchange, pigment level), nutritional quality (NPK) of plants and yield attributes (pod length, oil content, pods per plant, seeds per pod, 1000 seed weight and seed yield). The impact of aphids was not as prominent as in Experiment 2 (with beetles). This improvement was higher at late growth stage (75 DAS) as the aphid population decreased considerably. The results were much better in cultivar Alankar as compared to most susceptible one; Rohini. The improved defense mechanism was further supported with the higher level of proline accumulation in cultivar Alankar, as compared to cv. Rohini.

Experiment 4

This experiment was conducted to study the different concentrations of JA on the two selected cultivars of *Brassica juncea* cvs. Alankar and Rohini. The agricultural conditions and experimental design were same as in Experiment 1. Jasmonic acid (0, 0.5, 1.0 or 1.5 mM) was sprayed on the foliage of Alanakar and Rohini at 45 DAS. Plants were sampled at 60 and 75 DAS. No significant change in growth parameters (length, fresh and dry mass of shoot and root, area and number of leaves per plant) and nutritional quality (NPK level) was observed on either of the cultivar treated with 0.5 or 1.5 mM JA. Only treatment with 1.0 mM JA enhanced the growth and yield characteristics (seed yield and oil content) of two the cultivars, significantly. This response was more prominent in Alankar than in Rohini at late growth stage (75 DAS). Jasmonic acid application strongly stimulated the level of photosynthetic pigments (chlorophyll a, b, total chlorophyll and carotenoids content) and proline level. But, the level of proline enhanced was lesser as compared to that of aphid infested plants (Experiment 2). Jasmonic acid induced the stomatal closure in dose dependent manner in both the cultivars. This concentration of JA (0.1 mM) also increased the plant fresh and dry mass more effectively in Alankar as compared to Rohini. Larger number of beetles was attracted in JA treated Alankar than JA treated Rohini at 60 DAS.

Experiment 5

This experiment was laid with an objective to elucidate the effect of foliar spray of JA prior to aphid infestation on two selected cultivars of mustard (Alankar and Rohini). All cultivation practices, statistical and experimental design were same as in Experiment 1. Both the cultivars of *Brassica juncea*; Alankar and Rohini, were sprayed with 0.1 mM of JA solution at 45 DAS followed (5 days later) by aphid infestation (50, 100 or 150 aphids per plant). The population of aphids significantly reduced with the progressing age of the plant (60 to 75 DAS). This resulted into the improvement of growth (length, fresh and dry mass of shoot and root, area and number of leaves per plant), stomatal dynamics (relative stomatal closure index, number of stomata), photosynthetic performance (net photosynthetic rate, stomata gaseous exchange, pigments level), nutritional quality (NPK level) and yield attributes (pod length, oil content, pods per plant, seeds per pod, 1000 seed weight and seed

yield) of plants. Alankar excelled in its growth response and resistant to aphids as compared to Rohini. The increase in proline level in JA treated plants confirmed its defensive role and protective nature in aphid induced water stress in plants.

It emerged from the data of experiment 5, that JA treatment induced only marginal increase in plant growth. But, findings elucidates that JA treatment enhanced defensive and protective abilities of the cultivars. To elucidate the role of volatile chemicals in plant defense including beetle attraction and chemical deterrence to aphid, GC-MS analysis was carried out. Analysis revealed that volatile organic compounds (VOCs) viz. allyl isothioscynate and 3- Hexan-1-ol increased in cultivar Alankar treated with JA (0.1 mM) and aphids as compared to plants infested alone with aphids. In addition to these, morphological defense features, leaf trichome density, hypertrophied pods, induction of extra floral nectarines, stomata leaf surface features indicated that aphid induced damage and reciprocal plant defense responses and signaling extended up to third trophic level in mustard-aphid-beetle food chain.

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Appendix

APPENDIX

1. Reagents for determination of chlorophyll content

80% acetone

80% mL of acetone was mixed in 20 mL of DDW

2. Preparation of reagents for proline estimation

Sulpho-salicylic acid (3%)

3 g of sulpho-salicylic acid was dissolved in sufficient DDW and final volume was maintained to100 cm^3, by using DDW.

Acid ninhydrin solution

1.25 g of ninhydrin was dissolved in a mixture of warm, 30 cm^3 of glacial acetic acid and 6 M phosphoric acid (pH 1.0) with agitation till it got dissolved. It was stored at 4°Cand used within 24 h.

The 6M phosphoric acid was prepared by mixing 11.8 cm^3 of phosphoric acid with 8.2 cm^3 of DDW.

3. Preparation of reagents for protein estimation

Tri-chloroacetic acid (TCA) (5%)

5 ml of TCA was mixed with 95 ml of DDW.

1N NaOH

40 g of NaOH was dissolved in sufficient DDW and final volume was made upto 1000 ml, by using DDW.

Preparation of reagent C

Reagent A: 2% sodium carbonate (2g dissolved in 100 ml DDW) and 0.1N NaOH (4 g NaOH dissolved in 1000 ml) were mixed in the ratio 1:1.

Reagent B: 0.5% copper sulphate (500 mg $CuSO_4$ dissolved in 100 ml) and 1% sodium tartarate (1 g sodium tartarate dissolved in 100 ml DDW) were mixed in the ratio 1:1.

Reagent C: 50 ml of reagent A was mixed with 1 ml of reagent B, except omission of sodium hydroxide.

Folin's phenol reagent (1 N)

The reagent obtained from LobaChemie Pvt. Ltd. Mumbai, India was diluted with DDW in the ratio 1:2.

4. Reagents for leaf-NPK estimation

Molybdic acid reagent (2.5%)

6.25 g of ammonium molybdate was dissolved in 175 mL distilled water to which 75 mL of 10 N-sulphuricacid was added.

1-amino-2-naphthole-4-sulphonic acid

0.5 g 1-amino-2-naphthole-4-sulphonic acid was dissolved in 195 mL of 15 % sodium bisulphide solution to which 5 mL of 20 % sodium sulphate solution was added. The solution was kept in amber coloured bottle.

Sodium hydroxide solution (2.5 N)

100 g NaOH dissolved in sufficient DDW and final volume was maintained up to 1000 mL with DDW.

Sodium silicate solution (10%)

10 g sodium was silicate dissolved in sufficient DDW and final volume was maintained up to 100 mL with DDW.

5. Total phenol estimation

Folin–Ciocalteu reagent (1 N)

Prepare 1X (1N) Folin-Ciocalteu Reagent by diluting the supplied 2X (2 N) reagent 1:1 with DDW.

Sodium carbonate (20%)

20 g sodium bicarbonate was dissolved in sufficient DDW to make up final volume 100 ml.

Insoluble polyvinyl pyrrolidone

Insoluble polyvinyl pyrrolidonewas purchased from Sigma Aldrich.

Standard tannic acid solution (0.1mg/ml)

0.1 mg chemical grade tannic acid was dissolved in 1 ml of DDW.

Annexure

Table 1. Effect of aphid infestation (40 aphids per plant) on aphid population growth and plant height (cm) of different cultivars of *Brassica juncea* at 60 and 75 DAS

Treatment	Number of aphids		Plant height (cm)	
	60 DAS	75 DAS	60 DAS	75 DAS
Alankar	65e±3.0	85d±4.0	61.48a±3.07	78.79a±3.28
PJK	73d±3.0	75e±4.0	58.31ab±2.43	72.88ab±3.47
Varuna	84c±4.0	107c±5.0	53.84bc±2.56	67.58b±2.82
Sakha	95b±5.0	121b±5.0	50.13cd±2.28	61.24c±2.66
Rohini	105a±5.0	135a±6.0	46.02d±1.84	55.56c±2.31
LSD at 5%	6.617	7.809	4.329	5.410

Mean±SD, Different letters showing data in a column is significant at $p<0.05$

Table 2. Effect of aphid infestation (40 aphids per plant) on shoot length (cm) and root length (cm) of different cultivars of *Brassica juncea* at 60 and 75 DAS

Treatment	Shoot length (cm)		Root length (cm)	
	60 DAS	75 DAS	60 DAS	75 DAS
Alankar	46.72a±1.95	64.34a±2.68	15.86a±0.63	24.25a±1.10
PJK	44.45ab±1.85	60.41ab±2.42	14.72ab±0.74	22.47b±0.90
Varuna	41.17bc±1.79	55.67b±2.53	13.67b±0.59	19.11c±0.80
Sakha	37.80cd±1.51	50.71c±2.20	12.33c±0.51	17.84cd±0.78
Rohini	35.54d± 1.48	47.36c±2.15	11.98c±0.50	16.52d±0.75
LSD at 5%	3.300	4.476	1.100	1.619

Mean±SD, Different letters showing data in a column is significant at $p<0.05$

Table 3. Effect of aphid infestation (40 aphids per plant) on leaf number and leaf area (cm^2) of different cultivars of *Brassica juncea* at 60 and 75 DAS

Treatment	Leaf number		Leaf area (cm^2)	
	60 DAS	75 DAS	60 DAS	75 DAS
Alankar	40.03a±1.74	56.98a±2.37	33.82a±1.35	39.14a±1.96
PJK	38.67ab±1.84	53.26ab±2.32	30.33b±1.21	35.52b±1.69
Varuna	36.50bc±1.46	50.23bc±2.18	27.49c±1.15	31.12c±1.24
Sakha	34.76cd±1.39	47.11cd±1.96	25.45c±1.16	27.27d±1.24
Rohini	32.44d±1.41	44.96d±1.80	22.88d±0.92	23.61e±0.98
LSD at 5%	2.913	4.032	2.253	2.555

Mean±SD, Different letters showing data in a column is significant at $p<0.05$

Table 4. Effect of aphid infestation (40 aphids per plant) on plant fresh mass (g) and plant dry mass (g) of different cultivars of *Brassica juncea* at 60 and 75 DAS

Treatment	Plant fresh mass (g)		Plant dry mass (g)	
	60 DAS	75 DAS	60 DAS	75 DAS
Alankar	8.07a±0.38	10.92a±0.50	2.02a±0.09	2.72a±0.11
PJK	6.91b±0.33	10.02b±0.46	1.66b±0.08	2.54a±0.11
Varuna	5.82c±0.26	9.25c±0.40	1.44c±0.06	2.28b±0.11
Sakha	4.74d±0.19	8.38d±0.42	1.13d±0.05	2.02c±0.09
Rohini	4.13e±0.20	7.79d±0.34	1.04d±0.04	1.93c±0.08
LSD at 5%	0.492	0.746	0.121	0.185

Mean±SD, Different letters showing data in a column is significant at $p<0.05$

Table 5. Effect of aphid infestation (40 aphids per plant) on chlorophyll a and chlorophyll b of different cultivars of *Brassica juncea* at 60 and 75 DAS

Treatment	Chlorophyll a		Chlorophyll b	
	60 DAS	75 DAS	60 DAS	75 DAS
Alankar	1.080a±0.049	1.150a±0.048	0.463a±0.021	0.570a±0.024
PJK	0.940b±0.043	1.090ab±0.052	0.431ab±0.022	0.538ab±0.023
Varuna	0.860bc±0.043	1.040bc±0.050	0.397bc±0.020	0.504bc±0.022
Sakha	0.798c±0.032	0.989cd±0.049	0.365cd±0.016	0.474c±0.020
Rohini	0.708d±0.030	0.940d±0.045	0.327d±0.015	0.418d±0.021
LSD at 5%	0.071	0.083	0.032	0.040

Mean±SD, Different letters showing data in a column is significant at $p<0.05$

Table 6. Effect of aphid infestation (40 aphids per plant) on total chlorophyll and carotenoid level of different cultivars of *Brassica juncea* at 60 and 75 DAS

Treatment	Total chlorophyll		Carotenoid level	
	60 DAS	75 DAS	60 DAS	75 DAS
Alankar	1.543a±0.067	1.720a±0.082	0.352a±0.018	0.387a±0.017
PJK	1.371b±0.057	1.628ab±0.081	0.311b±0.012	0.324b±0.013
Varuna	1.257c±0.050	1.544bc±0.062	0.271c±0.013	0.295bc±0.013
Sakha	1.163c±0.048	1.463cd±0.064	0.242d±0.010	0.271cd±0.014
Rohini	1.035d±0.052	1.358d±0.057	0.232d±0.009	0.262d±0.012
LSD at 5%	0.102	0.123	0.022	0.024

Mean±SD, Different letters showing data in a column is significant at $p<0.05$

Table 7. Effect of aphid infestation (40 aphids per plant) on proline level (μ mol g^{-1} FM) and protein content (mg g^{-1} DM) contents of different cultivars of *Brassica juncea* at 60 and 75 DAS

Treatment	Proline content		Protein content	
	60 DAS	75 DAS	60 DAS	75 DAS
Alankar	15.20a±0.72	17.65a±0.84	19.82±0.90	20.32±0.81
PJK	14.05ab±0.61	16.89ab±0.73	19.49±0.89	19.94±0.95
Varuna	13.43bc±0.58	15.93bc±0.76	19.17±0.80	19.57±0.85
Sakha	12.67cd±0.51	15.33c±0.77	18.68±0.85	18.98±0.76
Rohini	12.08d±0.60	14.87c±0.68	18.19±0.76	18.43±0.80
LSD at 5%	1.075	1.284	NS	NS

Mean±SD, Different letters showing data in a column is significant at $p<0.05$

Table 8. Effect of aphid infestation (0, 50, 100 and 150 per plant) on shoot and root length (cm) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	Shoot length						Root length					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	51.67	42.33	47.00	71.44	56.67	64.06	17.16	13.76	15.46	26.78	19.11	22.95
50 aphids	46.24	35.45	40.85	63.20	46.87	55.04	15.74	11.87	13.81	23.97	16.25	20.11
100 aphids	41.23	30.85	36.04	55.65	40.18	47.91	14.19	10.71	12.45	21.62	14.47	18.04
150 aphids	36.23	27.25	31.74	46.66	35.12	40.89	12.97	9.42	11.19	19.26	12.67	15.97
Mean	43.84	33.97		59.23	44.71		15.02	11.44		22.91	15.63	
LSD at 5%												
Varieties		0.486			0.655			0.165			0.245	
Treatment		0.687			0.926			0.233			0.346	
Var. x Treat.		0.972			1.309			NS			0.489	

Table 9. Effect of aphid infestation (0, 50, 100 and 150 per plant) leaf number and area (cm^2) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	Leaf number per plant						Leaf area					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	44.75	37.23	40.99	58.41	47.76	53.08	40.63	35.34	37.99	47.14	38.99	43.07
50 aphids	39.41	32.02	35.72	51.41	40.60	46.00	33.44	19.73	26.59	38.25	21.38	29.82
100 aphids	34.83	26.89	30.86	44.29	33.06	38.68	31.09	16.75	23.92	35.52	17.96	26.74
150 aphids	30.26	22.72	26.49	39.20	27.71	33.46	29.59	14.66	22.12	33.62	15.75	24.68
Mean	37.31	29.72		48.33	37.28		33.69	21.62		38.63	23.52	
LSD at 5%												
Varieties		0.419			0.222			1.000			1.419	
Treatment		0.592			0.314			1.415			2.006	
Var. x Treat.		NS			0.444			2.001			2.857	

Sig = Significant; NS = Non-significant

Table 10. Effect of aphid infestation (0, 50, 100 and 150 per plant) on fresh and dry mass (g) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	Fresh mass						Dry mass					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	9.23	5.82	7.53	12.65	11.33	11.99	2.32	1.46	1.89	3.15	2.82	2.99
50 aphids	7.95	4.01	5.98	10.71	7.55	9.13	1.99	1.01	1.50	2.68	1.89	2.28
100 aphids	7.12	3.57	5.35	9.56	6.63	8.10	1.79	0.88	1.34	2.40	1.67	2.03
150 aphids	6.35	3.18	4.77	8.32	5.85	7.08	1.60	0.80	1.20	2.07	1.47	1.77
Mean	7.66	4.15		10.31	7.84		1.93	1.04		2.57	1.96	
LSD at 5%												
Varieties		0.156			0.229			0.039			0.057	
Treatment		0.221			0.323			0.056			0.081	
Var. x Treat.		0.313			0.457			0.079			0.114	

Table 11. Effect of aphid infestation (0, 50, 100 and 150 per plant) on chlorophyll a (mg g^{-1} FM) and chlorophyll b (mg g^{-1} FM) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	Chlorophyll a						Chlorophyll b					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	1.153	0.803	0.978	1.268	0.970	1.119	0.565	0.382	0.474	0.561	0.461	0.511
50 aphids	1.028	0.698	0.863	1.122	0.832	0.977	0.530	0.311	0.420	0.497	0.324	0.411
100 aphids	0.956	0.628	0.792	1.011	0.719	0.865	0.502	0.261	0.381	0.430	0.255	0.343
150 aphids	0.860	0.564	0.712	0.914	0.638	0.776	0.472	0.187	0.330	0.297	0.202	0.249
Mean	0.999	0.673		1.079	0.790		0.517	0.285		0.446	0.310	
LSD at 5%												
Varieties		0.013			0.005			0.015			0.014	
Treatment		0.018			0.007			0.021			0.020	
Var. x Treat.		0.026			0.011			0.030			0.028	

Sig = Significant; NS = Non-significant

Table 12. Effect of aphid infestation (0, 50, 100 and 150 per plant) on total chlorophyll level (mg g^{-1} FM) and carotenoid level (mg g^{-1} FM) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	Total chlorophyll level						Carotenoid level					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	1.712	1.183	1.448	1.825	1.428	1.627	0.513	0.377	0.445	0.584	0.447	0.516
50 aphids	1.550	1.002	1.276	1.616	1.151	1.384	0.343	0.222	0.283	0.377	0.255	0.316
100 aphids	1.453	0.885	1.169	1.437	0.968	1.203	0.326	0.202	0.264	0.362	0.231	0.297
150 aphids	1.329	0.747	1.038	1.218	0.837	1.028	0.307	0.187	0.247	0.343	0.212	0.278
Mean	1.511	0.954		1.524	1.096		0.372	0.247		0.417	0.286	
LSD at 5%												
Varieties		0.013			0.026			0.004			0.003	
Treatment		0.018			0.037			0.006			0.005	
Var. x Treat.		0.026			0.052			0.008			0.007	

Table 13. Effect of aphid infestation (0, 50, 100 and 150 per plant) on protein content (mg g^{-1} DM) and total phenol content (mg g^{-1} FM) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	Protein content						Total phenol					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	23.14	21.76	22.45	24.09	22.19	23.14	3.52	3.02	3.27	4.21	3.87	4.04
50 aphids	19.63	17.96	18.80	20.18	18.02	19.10	3.23	2.74	2.98	3.81	3.42	3.61
100 aphids	17.97	16.15	17.06	18.26	16.16	17.21	3.06	2.62	2.84	3.69	3.23	3.46
150 aphids	15.58	14.23	14.91	15.69	14.16	14.93	2.98	2.46	2.72	3.57	3.00	3.29
Mean	19.08	17.53		19.56	17.63		3.20	2.71		3.82	3.38	
LSD at 5%												
Varieties		0.130			0.133			0.019			0.055	
Treatment		0.184			0.188			0.027			0.078	
Var. x Treat.		0.261			0.266			0.039			0.111	

Sig = Significant; NS = Non-significant

Table 14. Effect of aphid infestation (0, 50, 100 and 150 per plant) on proline content (μ mol g^{-1} FM) and N content (mg g^{-1} DM) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	Proline content						N content					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	12.99	11.07	12.03	15.03	13.47	14.25	5.26	5.02	5.14	6.51	5.29	5.90
50 aphids	15.41	12.49	13.95	17.99	15.42	16.71	5.06	4.81	4.94	6.18	4.99	5.59
100 aphids	18.02	15.15	16.59	21.57	19.05	20.31	4.84	4.60	4.72	5.85	4.73	5.29
150 aphids	21.42	16.78	19.10	25.33	22.26	23.80	4.57	4.34	4.46	5.50	4.44	4.97
Mean	16.96	13.87		19.98	17.55		4.93	4.69		6.01	4.86	
LSD at 5%												
Varieties		0.662			0.320			0.005			0.039	
Treatment		0.936			0.453			0.007			0.055	
Var. x Treat.		1.324			0.641			0.010			0.078	

Table 15. Effect of aphid infestation (0, 50, 100 and 150 per plant) on P and K content (mg g^{-1} DM) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	P content						K content					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	11.10	9.22	10.16	12.85	10.07	11.46	6.03	9.92	7.98	7.41	12.80	10.11
50 aphids	10.21	8.15	9.18	11.67	8.84	10.26	6.34	10.36	8.35	7.99	13.68	10.84
100 aphids	9.23	7.46	8.35	10.32	7.84	9.08	6.69	10.92	8.81	8.43	14.59	11.51
150 aphids	8.25	6.72	7.49	9.17	7.04	8.11	6.82	11.12	8.97	8.55	14.63	11.59
Mean	9.70	7.89		11.00	8.45		6.47	10.58		8.10	13.93	
LSD at 5%												
Varieties		0.127			0.170			0.152			0.497	
Treatment		0.179			0.240			0.215			0.702	
Var. x Treat.		0.253			0.340			NS			NS	

Sig = Significant; NS = Non-significant

Table 16. Effect of aphid infestation (0, 50, 100 and 150 per plant) on number of stomata on abaxial and adaxial leaf surface of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	No. of stomata (Abaxial surface)						No. of stomata (Adaxial surface)					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	21.3	18.6	20.0	24.8	22.1	23.5	11.3	10.7	11.0	14.8	12.6	13.7
50 aphids	19.8	16.5	18.2	20.6	16.6	18.6	11.0	9.5	10.2	12.3	9.5	10.9
100 aphids	19.2	16.0	17.6	19.4	15.2	17.3	10.8	9.2	10.0	11.6	8.7	10.1
150 aphids	18.8	15.7	17.3	18.2	14.8	16.5	10.7	9.0	9.9	10.9	8.5	9.7
Mean	19.8	16.7		20.8	17.2		10.9	9.6		12.4	9.8	
LSD at 5%												
Varieties		0.103			0.163			0.174			0.192	
Treatment		0.146			0.230			0.276			0.271	
Var. x Treat.		0.207			0.325			0.348			0.373	

Table 17. Effect of aphid infestation (0, 50, 100 and 150 per plant) on relative stomatal closure index (RSCI) of abaxial and adaxial surface of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	RSCI (Abaxial leaf surface)						RSCI (Adaxial leaf surface)					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
50 aphids	0.56	0.59	0.57	0.88	0.91	0.89	0.55	0.57	0.56	0.86	0.88	0.87
100 aphids	0.62	0.66	0.64	0.91	0.95	0.93	0.60	0.63	0.61	0.88	0.91	0.89
150 aphids	0.68	0.73	0.70	0.94	0.98	0.96	0.65	0.69	0.67	0.90	0.96	0.93
Mean	0.62	0.66		0.91	0.94		0.60	0.63		0.88	0.92	
LSD at 5%												
Varieties		0.006			0.003			0.005			0.008	
Treatment		0.007			0.004			0.007			0.010	
Var. x Treat.		0.010			0.006			0.009			0.013	

Sig = Significant; NS = Non-significant

Table 18. Effect of aphid infestation (0, 50, 100 and 150 per plant) on net photosynthetic rate (P_N; μ mol CO_2 m^{-2} sec^{-1}) and stomatal conductance (g_s; mol m^{-2} sec^{-1}) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	Net photosynthetic rate (P_N)						Stomatal conductance (g_s)					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	10.32	9.20	9.76	12.53	11.07	11.80	40.11	34.82	37.47	46.64	38.47	42.56
50 aphids	9.20	7.44	8.32	9.80	7.35	8.58	33.01	19.44	26.23	37.38	21.10	29.24
100 aphids	7.69	5.57	6.63	8.09	5.34	6.72	30.69	16.50	23.60	34.21	17.34	25.78
150 aphids	6.80	4.59	5.70	6.89	3.83	5.36	29.21	14.44	21.83	32.33	14.00	23.17
Mean	8.50	6.70		9.33	6.90		33.26	21.30		37.64	22.73	
LSD at 5%												
Varieties		0.269			0.296			0.987			1.109	
Treatment		0.381			0.418			1.396			1.569	
Var. x Treat.		0.539			0.591			1.974			2.218	

Table 19. Effect of aphid infestation (0, 50, 100 and 150 per plant) on pod length (cm) and oil content (%) of *Brassica juncea* cvs. Alankar and Rohini at harvest (120 DAS)

Treatment	Pods length			Oil content		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	5.05	3.94	4.50	36.42	32.87	34.65
50 aphids	4.77	3.64	4.21	31.92	27.68	29.80
100 aphids	4.38	3.34	3.86	28.59	23.65	26.12
150 aphids	4.12	3.03	3.58	22.95	16.88	19.92
Mean	4.58	3.49		29.97	25.27	
LSD at 5%						
Varieties		0.021			0.621	
Treatment		0.029			0.878	
Var. x Treat.		0.041			1.242	

Sig = Significant; NS = Non-significant

Table 20. Effect of aphid infestation (0, 50, 100 and 150 per plant) on yield characteristics of *Brassica juncea* cvs. Alankar and Rohini at harvest (120 DAS)

Treatment	Pods per plant			Seeds per pod			1000 seeds weight (g)			Seed yield (g)		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	108.66	86.45	97.56	11.93	10.78	11.36	5.54	4.23	4.89	7.18	3.94	5.56
50 aphids	91.58	71.67	81.63	10.58	9.44	10.01	4.77	3.26	4.02	4.62	2.35	3.49
100 aphids	78.32	60.47	69.40	9.23	8.01	8.62	3.84	2.53	3.19	2.78	1.27	2.02
150 aphids	61.46	45.76	53.61	8.12	6.90	7.51	3.14	1.78	2.46	1.57	0.56	1.06
Mean	85.01	66.09		9.97	8.78		4.32	2.95		4.04	2.03	
LSD at 5%												
Varieties		1.599			0.023			0.055			0.143	
Treatment		2.622			0.033			0.078			0.202	
Var. x Treat.		3.199			0.046			0.111			0.286	

Table 21. Population count of aphid and number of beetle attracted after infestation (0, 50, 100 and 150 per plant) on *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	Aphid count						Number of beetles attracted					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	0	0	0	0	0	0	0	0	0	0	0	0
50 aphids	81	119	100	117	138	128	9	7	8	3	2	3
100 aphids	132	147	140	176	195	186	11	10	11	4	3	4
150 aphids	193	209	201	240	255	248	10	8	9	2	2	2
Mean	102	119		133	147		8	6		2	2	
LSD at 5%												
Varieties		4.539			4.811			0.288			0.087	
Treatment		6.419			6.804			0.408			0.123	
Var. x Treat.		9.077			9.622			0.577			0.174	

Sig = Significant; NS = Non-significant

Table 22. Responses of shoot and root length (cm) of *Brassica juncea* cvs. Alankar and Rohini exposed at 45 DAS to varying level of aphid infestations (0, 50, 100, 150 aphid per plant) and successive predation of aphids (5 days after) by 2 beetle per plant studied at 60 and 75 DAS

Treatment	Shoot length						Root length					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	52.04	42.62	47.33	71.73	57.02	64.38	17.58	14.04	15.81	27.11	19.33	23.22
50 aphids+2 beetle	48.36	37.23	42.79	65.50	48.79	57.15	16.79	13.17	14.98	25.66	17.64	21.65
100 aphids+2 beetle	44.55	34.02	39.29	60.91	43.41	52.16	15.60	11.21	13.41	23.68	14.97	19.32
150 aphids+2 beetle	41.31	29.29	35.30	54.72	36.56	45.64	14.50	9.72	12.11	21.94	12.98	17.46
Mean	46.57	35.79		63.21	46.44		16.12	12.03		24.60	16.23	
LSD at 5%												
Varieties		0.597			0.875			0.246			0.303	
Treatment		0.844			1.237			0.348			0.429	
Var. x Treat.		1.194			1.750			0.493			0.607	

Table 23. Responses of leaf number and area (cm^2) of *Brassica juncea* cvs. Alankar and Rohini exposed at 45 DAS to varying level of aphid infestations (0, 50, 100, 150 aphid per plant) and successive predation of aphids (5 days after) by 2 beetle per plant studied at 60 and 75 DAS

Treatment	Leaf number $plant^{-1}$						Leaf area					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	44.13	37.48	40.81	58.72	48.07	53.40	40.85	35.59	38.22	47.32	39.18	43.25
50 aphids+ 2 beetle	40.46	32.68	36.57	53.05	41.22	47.14	36.17	29.70	32.94	41.00	31.82	36.41
100 aphids+2 beetle	37.23	30.01	33.62	48.81	37.13	42.97	33.00	27.12	30.06	37.18	28.99	33.08
150 aphids+2 beetle	33.60	26.62	30.11	43.99	33.19	38.59	29.40	22.50	25.95	32.35	23.57	27.96
Mean	38.85	31.70		51.14	39.90		34.86	28.73		39.46	30.89	
LSD at 5%												
Varieties		1.015			0.330			0.393			0.279	
Treatment		1.436			0.466			0.556			0.395	
Var. x Treat.		NS			0.659			0.786			0.559	

Sig = Significant; NS = Non-significant

Table 24 Responses of fresh and dry mass (g) of *Brassica juncea* cvs. Alankar and Rohini exposed at 45 DAS to varying level of aphid infestations (0, 50, 100, 150 aphid per plant) and successive predation of aphids (5 days after) by 2 beetle per plant studied at 60 and 75 DAS

Treatment	Fresh mass (g)						Dry mass (g)					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	9.07	5.64	7.36	12.46	11.12	11.79	2.41	1.56	1.99	3.26	2.91	3.09
50 aphids+2 beetle	8.06	4.76	6.41	10.81	9.10	9.95	2.13	1.32	1.73	2.84	2.39	2.62
100 aphids+2 beetle	7.69	4.55	6.12	10.30	8.73	9.52	2.03	1.26	1.65	2.71	2.30	2.50
150 aphids+2 beetle	7.53	4.44	5.98	10.02	8.46	9.24	1.98	1.23	1.61	2.64	2.23	2.43
Mean	8.09	4.85		10.90	9.35		2.14	1.34		2.86	2.46	
LSD at 5%												
Varieties		0.089			0.086			0.026			0.023	
Treatment		0.126			0.122			0.036			0.032	
Var. x Treat.		0.178			0.172			0.052			0.045	

Table 25. Responses Chlorophyll a and b content (mg g^{-1} FM) of *Brassica juncea* cvs. Alankar and Rohini exposed at 45 DAS to varying level of aphid infestations (0, 50, 100, 150 aphid per plant) and successive predation of aphids (5 days after) by 2 beetle per plant studied at 60 and 75 DAS

Treatment	Chlorophyll a						Chlorophyll b					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	1.151	0.802	0.977	1.263	0.971	1.117	0.610	0.230	0.420	0.740	0.350	0.545
50 aphids+2 beetle	1.044	0.627	0.836	1.136	0.721	0.929	0.538	0.280	0.409	0.626	0.262	0.444
100 aphids+2 beetle	0.970	0.517	0.743	1.031	0.600	0.816	0.471	0.303	0.387	0.557	0.230	0.393
150 aphids+2 beetle	0.883	0.426	0.655	0.945	0.486	0.715	0.426	0.322	0.374	0.492	0.200	0.346
Mean	1.012	0.593		1.094	0.694		0.511	0.284		0.604	0.260	
LSD at 5%												
Varieties		0.029			0.032			0.015			0.017	
Treatment		0.041			0.046			0.021			0.024	
Var. x Treat.		0.059			0.065			0.029			0.033	

Sig = Significant; NS = Non-significant

Table 26. Responses of total chlorophyll and carotenoid level (mg g^{-1} FM) of *Brassica juncea* cvs. Alankar and Rohini exposed at 45 DAS to varying level of aphid infestations (0, 50, 100, 150 aphid per plant) and successive predation of aphids (5 days after) by 2 beetle per plant studied at 60 and 75 DAS

Treatment	Total chlorophyll						Carotenoid level					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	1.757	1.031	1.394	2.002	1.322	1.662	0.530	0.360	0.445	0.570	0.470	0.520
50 aphids+2 beetle	1.578	0.906	1.242	1.762	0.982	1.372	0.400	0.261	0.331	0.426	0.335	0.381
100 aphids+2 beetle	1.442	0.818	1.130	1.588	0.829	1.208	0.384	0.249	0.316	0.406	0.320	0.363
150 aphids+2 beetle	1.310	0.746	1.028	1.432	0.686	1.059	0.373	0.243	0.308	0.395	0.312	0.353
Mean	1.522	0.875		1.696	0.955		0.422	0.278		0.449	0.359	
LSD at 5%												
Varieties		0.036			0.024			0.010			0.004	
Treatment		0.051			0.035			0.015			0.006	
Var. x Treat.		0.073			0.049			0.021			0.009	

Table 27. Responses of protein(mg g^{-1} DM) and total phenol content of *Brassica juncea* cvs. Alankar and Rohini exposed at 45 DAS to varying level of aphid infestations (0, 50, 100, 150 aphid per plant) and successive predation of aphids (5 days after) by 2 beetle per plant studied at 60 and 75 DAS

Treatment	Protein content						Total phenol					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	23.32	21.94	22.63	24.28	22.37	23.33	3.73	3.21	3.47	4.53	4.05	4.29
50 aphids+2 beetle	20.11	18.44	19.27	21.47	19.58	20.52	3.44	3.01	3.22	4.24	3.72	3.98
100 aphids+2 beetle	18.64	17.02	17.83	20.06	17.95	19.01	3.30	2.82	3.06	4.07	3.51	3.79
150 aphids+2 beetle	16.36	14.90	15.63	18.26	15.73	17.00	3.20	2.68	2.94	3.86	3.35	3.60
Mean	19.61	18.07		21.02	18.91		3.42	2.93		4.18	3.66	
LSD at 5%												
Varieties		0.078			0.170			0.025			0.019	
Treatment		0.111			0.241			0.035			0.027	
Var. x Treat.		0.157			0.340			0.049			0.038	

Sig = Significant; NS = Non-significant

Table 28. Responses of proline content (μ mol g^{-1} FM) and N content (mg g^{-1} DM) of *Brassica juncea* cvs. Alankar and Rohini exposed at 45 DAS to varying level of aphid infestations (0, 50, 100, 150 aphid per plant) and successive predation of aphids (5 days after) by 2 beetle per plant studied at 60 & 75 DAS

Treatment	Proline content						N content					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	13.27	11.36	12.32	15.34	13.76	14.55	5.37	5.14	5.26	6.60	5.38	5.99
50 aphids+2 beetle	15.05	12.26	13.65	16.91	14.34	15.62	5.21	4.94	5.08	6.50	5.16	5.83
100 aphids+2 beetle	17.57	13.17	15.37	19.74	15.33	17.54	5.02	4.75	4.89	6.24	4.89	5.57
150 aphids+2 beetle	21.25	14.91	18.08	23.61	18.42	21.01	4.76	4.62	4.69	5.93	4.59	5.26
Mean	16.78	12.92		18.90	15.46		5.09	4.86		6.32	5.00	
LSD at 5%												
Varieties		0.512			0.732			0.035			0.033	
Treatment		0.724			1.035			0.049			0.046	
Var. x Treat.		1.024			1.464			0.070			0.065	

Table 29 Responses of P and K content (mg g^{-1} DM) of *Brassica juncea* cvs. Alankar and Rohini exposed at 45 DAS to varying level of aphid infestations (0, 50, 100, 150 aphid per plant) and successive predation of aphids (5 days after) by 2 beetle per plant studied at 60 and 75 DAS

Treatment	P content						K content					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	11.21	9.34	10.28	12.93	10.17	11.55	6.10	9.96	8.03	7.47	12.87	10.17
50 aphids+2 beetle	10.40	8.45	9.42	12.24	9.42	10.83	6.42	10.37	8.40	7.82	13.30	10.56
100 aphids+2 beetle	9.44	7.66	8.55	11.17	8.55	9.86	6.75	10.90	8.82	8.21	13.97	11.09
150 aphids+2 beetle	8.46	6.84	7.65	10.03	7.67	8.85	6.87	11.09	8.98	8.35	14.20	11.28
Mean	9.88	8.07		11.59	8.95		6.54	10.58		7.96	13.59	
LSD at 5%												
Varieties		0.082			0.119			0.381			0.323	
Treatment		0.115			0.169			0.541			0.456	
Var. x Treat.		0.163			0.238			NS			NS	

Sig = Significant; NS = Non-significant

Table 30. Responses of number of stomata on abaxial and adaxial surface of *Brassica juncea* cvs. Alankar and Rohini exposed at 45 DAS to varying level of aphid infestations (0, 50, 100, 150 aphid per plant) and successive predation of aphids (5 days after) by 2 beetle per plant studied at 60 and 75 DAS

Treatment	No. of stomata (Abaxial surface) L						No. of stomata (Adaxial surface) U					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	21.5	18.7	20.1	24.6	22.6	23.6	11.4	10.8	11.1	14.8	12.7	13.8
50 aphids+2 beetle	20.6	16.9	18.8	24.1	22.1	23.1	10.8	9.7	10.3	14.5	12.3	13.4
100 aphids+2 beetle	20.2	16.5	18.3	24.0	22.0	23.0	10.6	9.5	10.1	14.4	12.3	13.3
150 aphids+2 beetle	19.6	16.2	17.9	23.9	21.9	22.9	10.3	9.4	9.9	14.3	12.2	13.2
Mean	20.5	17.1		24.1	22.1		10.8	9.9		14.5	12.4	
LSD at 5%												
Varieties		0.246			0.260			0.138			0.152	
Treatment		0.348			0.360			0.195			0.215	
Var. x Treat.		0.492			NS			0.276			NS	

Table 31. Responses of relative stomatal closure index (RSCI) of abaxial and adaxial surface of *Brassica juncea* cvs. Alankar and Rohini exposed at 45 DAS to varying level of aphid infestations (0, 50, 100, 150 aphid per plant) and successive predation of aphids (5 days after) by 2 beetle per plant studied at 60 and 75 DAS

Treatment	RSCI (Abaxial leaf surface)						RSCI (Adaxial leaf surface)					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
50 aphids+2 beetle	0.51	0.51	0.51	0.81	0.83	0.82	0.50	0.50	0.65	0.80	0.80	0.40
100 aphids+2 beetle	0.55	0.56	0.56	0.83	0.85	0.84	0.54	0.55	0.69	0.81	0.83	0.42
150 aphids+2 beetle	0.60	0.62	0.61	0.85	0.88	0.87	0.59	0.60	0.73	0.83	0.86	0.43
Mean	0.55	0.56		0.83	0.85		0.54	0.55		0.81	0.83	
LSD at 5%												
Varieties		0.030			0.004			0.006			0.007	
Treatment		0.037			0.004			0.007			0.009	
Var. x Treat.		0.053			0.006			0.010			0.012	

Sig = Significant; NS = Non-significant

Table 32. Responses of net photosynthetic rate (P_N; μ mol CO_2 m^{-2} sec^{-1}) and stomatal conductance (g_s; m mol m^{-2} sec^{-1}) *Brassica juncea* cvs. Alankar and Rohini exposed at 45 DAS to varying level of aphid infestations (0, 50, 100, 150 aphid per plant) and successive predation of aphids (5 days after) by 2 beetle per plant studied at 60 and 75 DAS

Treatment	Net photosynthetic rate (P_N)						Stomatal conductance (g_s)					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	10.37	9.29	9.83	12.61	11.15	11.88	39.51	34.99	37.25	46.72	40.32	43.52
50 aphids+2 beetle	10.73	9.49	10.11	13.22	11.44	12.33	34.38	30.22	32.30	38.99	31.94	35.47
100 aphids+2 beetle	9.80	8.70	9.25	12.85	10.76	11.81	31.92	27.41	29.67	35.60	29.03	32.32
150 aphids+2 beetle	8.31	7.27	7.79	10.97	9.27	10.12	28.43	23.84	26.14	29.54	23.05	26.30
Mean	9.80	8.69		12.41	10.66		33.56	29.12		37.71	31.09	
LSD at 5%												
Varieties		0.050			0.132			0.166			0.360	
Treatment		0.070			0.186			0.265			0.509	
Var. x Treat.		0.100			0.264			0.333			NS	

Table 33. Responsesof pod length (cm) and oil content (%) of *Brassica juncea* cvs. Alankar and Rohini exposed at 45 DAS to varying level of aphid infestations (0, 50, 100, 150 aphid per plant) and successive predation of aphids (5 days after) by 2 beetle per plant studied at 60 and 75 DAS

	Pods length			Oil content		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	5.06	3.93	4.50	36.54	33.13	34.84
50 aphids+2 beetle	4.80	3.65	4.23	29.40	32.96	31.18
100 aphids+2 beetle	4.41	3.35	3.88	30.15	26.02	28.09
150 aphids+2 beetle	4.15	3.04	3.59	25.22	19.90	22.56
Mean	4.61	3.49		30.33	28.00	
LSD at 5%						
Varieties		0.047			0.460	
Treatment		0.066			0.650	
Var. x Treat.		NS			0.919	

Sig = Significant; NS = Non-significant

Table 34. Responsesof yield characteristics of *Brassica juncea* cvs. Alankar and Rohini exposed at 45 DAS to varying level of aphid infestations (0, 50, 100, 150 aphid per plant) and successive predation of aphids (5 days after) by 2 beetle per plant studied at 60 and 75 DAS

	Pods per plant			Seeds per pod			1000 seeds weight (g)			Seed yield (g)		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	110.64	88.44	99.54	11.91	10.76	11.34	5.52	4.24	4.88	7.27	4.03	5.65
50 aphids+2 beetle	96.01	75.40	85.71	10.69	9.54	10.11	4.57	3.40	3.98	4.69	2.45	3.57
100 aphids+2 beetle	82.27	64.45	73.36	9.11	8.01	8.56	4.19	3.04	3.62	3.14	1.57	2.36
150 aphids+2 beetle	67.26	49.33	58.29	7.87	6.78	7.32	3.54	2.41	2.97	1.87	0.81	1.34
Mean	89.05	69.41		9.89	8.77		4.45	3.27		4.24	2.21	
LSD at 5%												
Varieties		1.168			0.019			0.038			0.134	
Treatment		1.652			0.026			0.054			0.190	
Var. x Treat.		2.337			0.037			0.076			0.268	

Table 35. Responses of aphid populations on *Brassica juncea* cvs. Alankar and Rohini exposed at 45 DAS to varying level of aphid infestations (0, 50, 100, 150 aphid per plant) and successive predation of aphids (5 days after) by 2 beetle per plant at 60 and 75 DAS

	Aphid count					
	60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	0	0	0	0	0	0
50 aphids+2 beetle	58	63	61	96	113	105
100 aphids+2 beetle	107	116	112	153	168	161
150 aphids+2 beetle	171	181	176	220	231	226
Mean	84	90		117	128	
LSD at 5%						
Varieties		2.633			4.380	
Treatment		3.723			6.195	
Var. x Treat.		5.266			8.761	

Sig = Significant; NS = Non-significant

Table 36. Effect of jasmonic acid (0.5, 1.0 and 1.5 mM) on shoot and root length (cm) of *Brassica juncea* cv. Alankar and Rohini at 60 and 75 DAS

Treatment	Shoot length						Root length					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	51.32	42.07	46.70	71.21	56.42	63.77	16.92	13.54	15.24	26.55	18.88	22.72
JA (0.5mM)	53.73	42.94	48.33	74.81	58.48	66.65	17.31	13.66	15.48	27.26	19.10	23.18
JA (1.0mM)	55.80	43.67	49.73	77.89	59.76	68.82	17.42	13.78	15.60	27.48	19.31	23.40
JA (1.5mM)	53.34	42.50	47.92	74.27	57.51	65.89	17.09	13.57	15.33	26.90	18.98	22.94
Mean	53.55	42.80		74.52	58.04		17.19	13.64		27.05	19.07	
LSD at 5%												
Varieties		0.689			0.761			0.189			0.139	
Treatment		0.974			1.077			0.267			0.196	
Var. x Treat.		1.378			1.523			NS			0.278	

Table 37. Effect of jasmonic acid (0.5, 1.0 and 1.5 mM) on leaf number and area (cm^2) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	Leaf number plant^{-1}						Leaf area					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	44.56	37.08	40.82	58.18	47.53	52.86	40.41	35.10	37.76	46.92	38.75	42.84
JA (0.5mM)	45.61	37.74	41.67	59.84	48.47	54.16	41.07	35.36	38.22	47.81	39.18	43.49
JA (1.0mM)	46.07	38.17	42.12	60.44	49.08	54.76	41.25	35.43	38.34	47.97	39.30	43.63
JA (1.5mM)	45.20	37.47	41.34	59.23	48.18	53.71	40.89	35.27	38.08	47.64	39.13	43.38
Mean	45.36	37.61		59.42	48.32		40.91	35.29		47.58	39.09	
LSD at 5%												
Varieties		0.644			0.184			0.185			0.123	
Treatment		0.910			0.261			0.261			0.174	
Var. x Treat.		NS			0.369			NS			0.247	

Sig = Significant; NS = Non-significant

Table 38. Effect of jasmonic acid (0.5, 1.0 and 1.5 mM) on fresh and dry mass (g) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	Fresh mass						Dry mass					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	9.05	5.41	7.23	12.47	11.12	11.80	2.25	1.37	1.81	3.06	2.75	2.91
JA (0.5mM)	9.55	5.60	7.57	13.27	11.63	12.45	2.35	1.42	1.89	3.28	2.88	3.08
JA (1.0mM)	9.66	5.66	7.66	13.43	11.68	12.56	2.39	1.44	1.91	3.31	2.90	3.10
JA (1.5mM)	9.47	5.59	7.53	13.21	11.48	12.35	2.35	1.41	1.88	3.25	2.85	3.05
Mean	9.43	5.56		13.10	11.48		2.34	1.41		3.22	2.85	
LSD at 5%												
Varieties		0.018			0.027			0.090			0.104	
Treatment		0.025			0.038			0.127			0.148	
Var. x Treat.		0.035			0.054			0.180			0.209	

Table 39. Effect of jasmonic acid (0.5, 1.0 and 1.5 mM) on chlorophyll a and b (mg g^{-1} FM) level of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	Chlorophyll a						Chlorophyll b					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	1.157	0.805	0.981	1.270	0.973	1.122	0.566	0.374	0.470	0.567	0.469	0.518
JA (0.5mM)	1.359	0.919	1.139	1.529	1.132	1.330	0.678	0.428	0.553	0.691	0.548	0.620
JA (1.0mM)	1.399	0.932	1.165	1.576	1.155	1.365	0.708	0.449	0.579	0.723	0.577	0.650
JA (1.5mM)	1.317	0.877	1.097	1.477	1.091	1.284	0.653	0.412	0.533	0.668	0.529	0.599
Mean	1.308	0.883		1.463	1.088		0.651	0.416		0.662	0.531	
LSD at 5%												
Varieties		0.028			0.031			0.014			0.013	
Treatment		0.039			0.044			0.019			0.018	
Var. x Treat.		0.056			0.062			0.027			0.026	

Sig = Significant; NS = Non-significant

Table 40. Effect of jasmonic acid (0.5, 1.0 and 1.5 mM) on total chlorophyll level (mg g^{-1} FM) and carotenoid level (mg g^{-1} FM) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	Total chlorophyll level						Carotenoid level					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	1.721	1.176	1.449	1.837	1.441	1.639	0.570	0.430	0.500	0.540	0.350	0.445
JA (0.5mM)	2.034	1.345	1.689	2.218	1.678	1.948	0.743	0.531	0.637	0.683	0.419	0.551
JA (1.0mM)	2.104	1.380	1.742	2.298	1.730	2.014	0.760	0.545	0.653	0.698	0.432	0.565
JA (1.5mM)	1.967	1.287	1.627	2.146	1.618	1.882	0.729	0.506	0.618	0.670	0.401	0.535
Mean	1.956	1.297		2.125	1.617		0.701	0.503		0.648	0.401	
LSD at 5%												
Varieties		0.045			0.044			0.021			0.019	
Treatment		0.064			0.062			0.030			0.027	
Var. x Treat.		0.091			0.088			0.043			0.038	

Table 41. Effect of jasmonic acid (0.5, 1.0 and 1.5 mM) on protein content (mg g^{-1} DM) and total phenol content of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	Protein content						Total phenol					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	22.96	21.54	22.25	23.92	21.97	22.95	3.35	2.85	3.10	4.04	3.69	3.87
JA (0.5mM)	23.08	21.34	22.21	24.49	22.02	23.25	3.45	2.87	3.16	4.22	3.78	4.00
JA (1.0mM)	23.31	21.71	22.51	25.19	22.32	23.75	3.54	2.93	3.23	4.31	3.87	4.09
JA (1.5mM)	22.62	21.05	21.83	24.26	21.58	22.92	3.28	2.82	3.05	3.98	3.72	3.85
Mean	22.99	21.41		24.46	21.97		3.40	2.87		4.14	3.77	
LSD at 5%												
Varieties		0.075			0.196			0.036			0.045	
Treatment		0.106			0.278			0.050			0.063	
Var. x Treat.		0.150			0.393			0.071			0.089	

Sig = Significant; NS = Non-significance

Table 42. Effect of jasmonic acid (0.5, 1.0 and 1.5 mM) on proline content (μ mol g^{-1} FM) and N content (mg g^{-1} DM) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	Proline content						N content					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	12.65	10.75	11.70	14.71	13.14	13.93	5.12	4.96	5.04	6.44	5.22	5.83
JA (0.5mM)	13.87	11.24	12.55	15.77	13.43	14.60	5.24	5.05	5.14	6.68	5.32	6.00
JA (1.0mM)	16.12	12.23	14.17	18.31	14.58	16.44	5.27	5.07	5.17	6.72	5.35	6.03
JA (1.5mM)	19.21	13.60	16.40	21.28	15.61	18.44	5.22	5.02	5.12	6.61	5.29	5.95
Mean	15.46	11.95		17.52	14.19		5.21	5.02		6.61	5.29	
LSD at 5%												
Varieties		0.471			0.540			0.011			0.004	
Treatment		0.666			0.763			0.015			0.048	
Var. x Treat.		0.942			1.080			0.021			0.068	

Table 43. Effect of jasmonic acid (0.5, 1.0 and 1.5 mM) on P and K content (mg g^{-1} DM) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	P content						K content					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	11.03	9.15	10.09	12.77	9.99	11.38	6.00	9.91	7.96	7.40	12.79	10.10
JA (0.5mM)	11.11	9.17	10.14	12.94	10.06	11.50	6.30	10.10	8.20	7.72	12.93	10.32
JA (1.0mM)	11.17	9.24	10.20	13.04	10.19	11.62	6.37	10.16	8.27	7.75	12.96	10.36
JA (1.5mM)	10.97	9.07	10.02	12.81	9.95	11.38	6.08	10.04	8.06	7.47	12.92	10.20
Mean	11.07	9.16		12.89	10.05		6.19	10.05		7.59	12.90	
LSD at 5%												
Varieties		0.016			0.025			0.145			0.123	
Treatment		0.022			0.035			0.205			0.174	
Var. x Treat.		0.031			0.049			NS			NS	

Sig Significant; NS = Non-significant

Table 44. Effect of jasmonic acid (0.5, 1.0 and 1.5 mM) on number of stomata on abaxial and adaxial leaf surface of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	No. of stomata (Abaxial surface)						No. of stomata (Adaxial surface)					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	21.5	18.6	20.1	24.5	22.8	23.7	11.4	10.8	11.1	14.6	12.6	13.6
JA (0.5mM)	21.5	18.8	20.2	24.5	22.9	23.7	11.4	10.9	11.2	14.7	12.8	13.7
JA (1.0mM)	21.6	18.9	20.3	24.6	23.0	23.8	11.5	11.1	11.3	14.8	12.8	13.8
JA (1.5mM)	21.6	18.8	20.2	24.6	23.0	23.8	11.4	10.9	11.1	14.7	12.8	13.8
Mean	21.6	18.8		24.5	22.9		11.4	10.9		14.7	12.8	
LSD at 5%												
Varieties		0.683			0.798			0.38			0.47	
Treatment		NS			NS			NS			NS	
Var. x Treat.		NS			NS			NS			NS	

Table 45. Effect of jasmonic acid (0.5, 1.0 and 1.5 mM) on relative stomatal closure index (RSCI) of abaxial and adaxial surface of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	RSCI (Abaxial leaf surface)						RSCI (Adaxial leaf surface)					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
JA (0.5mM)	0.53	0.57	0.55	0.46	0.49	0.48	0.66	0.75	0.71	0.61	0.84	0.73
JA (1.0mM)	0.49	0.50	0.49	0.43	0.45	0.44	0.63	0.68	0.66	0.58	0.79	0.69
JA (1.5mM)	0.56	0.61	0.58	0.48	0.52	0.50	0.69	0.79	0.74	0.63	0.87	.0.75
Mean	0.53	0.56		0.46	0.49		0.66	0.74		0.61	0.83	
LSD at 5%												
Varieties		0.008			0.005			0.008			0.008	
Treatment		0.010			0.006			0.009			0.010	
Var. x Treat.		0.014			0.009			0.013			0.014	

Sig = Significant; NS = Non-significant

Table 46. Effect of jasmonic acid (0.5, 1.0 and 1.5 mM) on net photosynthetic rate (P_N; μ mol CO_2 m^{-2} sec^{-1}) and stomatal conductance (g_s; mol m^{-2} sec^{-1}) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	Net photosynthetic rate (P_N)						Stomatal conductance (g_s)					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	10.15	8.97	9.56	12.41	10.95	11.68	40.02	34.82	37.42	46.64	38.32	42.48
JA (0.5mM)	11.06	9.57	10.32	13.45	11.51	12.48	40.78	35.21	38.00	47.40	38.61	43.01
JA (1.0mM)	11.39	9.77	10.58	13.90	11.63	12.77	40.91	35.31	38.11	47.61	38.68	43.15
JA (1.5mM)	11.27	9.76	10.52	13.61	11.69	12.65	40.64	35.16	37.90	47.20	38.50	42.85
Mean	10.97	9.52		13.34	11.44		40.59	35.13		47.21	38.53	
LSD at 5%												
Varieties		0.087			0.192			0.105			0.153	
Treatment		0.123			0.271			0.149			0.216	
Var. x Treat.		0.174			0.384			0.211			0.305	

Table 47. Effect of jasmonic acid (0.5, 1.0 and 1.5 mM) on pod length (cm) and oil content (%) of *Brassica juncea* cvs. Alankar and Rohini at harvest (120 DAS)

	Pods length (cm)			Oil content (%)		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	5.04	3.92	4.48	36.45	32.89	34.67
JA (0.5mM)	5.14	3.96	4.55	39.12	34.59	36.86
JA (1.0mM)	5.15	3.98	4.56	40.01	35.29	37.65
JA (1.5mM)	5.12	3.95	4.54	38.43	34.05	36.24
Mean	5.11	3.95		38.50	34.21	
LSD at 5%						
Varieties		0.039			0.294	
Treatment		0.055			0.416	
Var. x Treat.		NS			0.588	

Sig = Significant; NS = Non-significant

Table 48. Effect of jasmonic acid (0.5, 1.0 and 1.5 mM) on yield characteristics of *Brassic juncea* cvs. Alankar and Rohini at harvest (120 DAS)

	Pods plant^{-1}			Seeds pod^{-1}			1000 seeds weight (g)			Seed yield (g)		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	106.62	84.41	95.52	11.95	10.79	11.37	5.57	4.26	4.92	7.10	3.88	5.49
JA (0.5mM)	107.73	85.05	96.39	12.17	10.93	11.55	5.68	4.29	4.99	7.45	3.99	5.72
JA (1.0mM)	108.53	85.61	97.07	12.26	11.03	11.64	5.71	4.34	5.03	7.60	4.10	5.85
JA (1.5mM)	107.62	84.54	96.08	12.09	10.87	11.48	5.66	4.27	4.97	7.37	3.93	5.65
Mean	107.62	84.90		12.12	10.91		5.66	4.29		7.38	3.97	
LSD at 5%												
Varieties		0.220			0.020			0.021			0.069	
Treatment		0.311			0.029			0.030			0.098	
Var. x Treat.		0.440			0.041			0.043			0.139	

Table 49. Effect of jasmonic acid (0.5, 1.0 and 1.5 mM) on number of beetles attracted on *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

	Number of beetles attracted					
	60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	0	0	0	0	0	0
JA (0.5mM)	12	9	11	4	3	4
JA (1.0mM)	14	11	13	6	5	6
JA (1.5mM)	10	9	10	5	3	4
Mean	9	7		4	3	
LSD at 5%						
Varieties		0.345			0.139	
Treatment		0.487			0.179	
Var. x Treat.		0.689			0.279	

Sig = Significant; NS = Non-significant

Table 50. Combined effect of jasmonic acid (1.0mM) and aphid infestation (50, 100 and 150 aphids per plant) on root and shoot length (cm) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	Shoot length						Root length					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	52.08	42.72	47.40	71.86	56.06	63.96	17.52	13.15	15.34	28.07	19.53	23.80
JA + 50 aphids	53.21	43.15	48.18	74.17	57.16	65.67	17.81	13.27	15.54	28.37	19.58	23.98
JA + 100 aphids	52.36	42.16	47.26	72.69	55.56	64.13	17.65	13.06	15.36	28.36	19.23	23.79
JA + 150 aphids	50.30	40.48	45.39	70.13	53.80	61.96	17.16	12.57	14.86	27.64	18.91	23.28
Mean	51.99	42.13		72.21	55.65		17.53	13.01		28.11	19.31	
LSD at 5%												
Varieties		0.212			0.316			0.265			0.139	
Treatment		0.301			0.446			0.375			0.196	
Var. x Treat.		0.425			0.631			NS			0.277	

Table 51. Combined effect of jasmonic acid (1.0 mM) and aphid infestation (50, 100 and 150 aphids per plant) on root and shoot length (cm) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	Leaf number per plant						Leaf area					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	45.06	37.61	41.34	58.83	48.13	53.48	40.95	35.62	38.29	47.53	39.36	43.45
JA + 50 aphids	44.82	36.55	40.68	58.72	47.27	53.00	40.64	35.11	37.87	47.35	38.90	43.12
JA + 100 aphids	44.58	36.45	40.51	58.52	46.95	52.74	40.40	34.86	37.63	47.05	38.63	42.84
JA + 150 aphids	44.22	35.51	39.86	58.09	46.29	52.19	40.03	34.60	37.32	46.69	38.42	42.56
Mean	44.67	36.53		58.54	47.16		40.50	35.05		47.15	38.83	
LSD at 5%												
Varieties		0.693			0.258			0.427			0.073	
Treatment		0.980			0.365			0.604			0.104	
Var. x Treat.		0.517			NS			0.147			NS	

Sig = Significant; NS = Non-significant

Table 52. Combined effect of jasmonic acid (1.0 mM) and aphid infestation (50, 100 and 150 aphids per plant) on fresh and dry mass (g) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	Fresh mass						Dry mass					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	9.52	6.23	7.88	13.93	11.67	12.80	2.49	1.57	2.03	3.31	2.92	3.12
JA + 50 aphids	8.84	5.59	7.21	12.92	10.51	11.71	2.30	1.40	1.85	3.09	2.64	2.87
JA + 100 aphids	8.36	5.26	6.81	12.32	10.02	11.17	2.18	1.33	1.76	2.94	2.51	2.73
JA + 150 aphids	7.95	4.99	6.47	11.78	9.46	10.62	2.08	1.26	1.67	2.80	2.38	2.59
Mean	8.67	5.52		12.74	10.41		2.26	1.39		3.03	2.61	
LSD at 5%												
Varieties		0.084			0.036			0.022			0.014	
Treatment		0.119			0.051			0.031			0.019	
Var. x Treat.		0.168			0.072			0.044			0.027	

Table 53. Combined effect of jasmonic acid (1.0 mM) and aphid infestation (50, 100 and 150 aphids per plant) on chlorophyll a and b (mg g^{-1} FM) level of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	Chlorophyll a						Chlorophyll b					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	1.155	0.806	0.981	1.270	0.967	1.116	0.566	0.380	0.473	0.563	0.468	0.516
JA + 50 aphids	1.092	0.713	0.903	1.238	0.870	1.054	0.508	0.323	0.416	0.523	0.421	0.472
JA + 100 aphids	1.072	0.694	0.883	1.222	0.849	1.035	0.488	0.305	0.397	0.510	0.402	0.456
JA + 150 aphids	1.049	0.652	0.851	1.197	0.795	0.996	0.465	0.276	0.371	0.499	0.382	0.440
Mean	1.092	0.716		1.230	0.870		0.507	0.321		0.524	0.418	
LSD at 5%												
Varieties		0.011			0.018			0.001			0.005	
Treatment		0.015			0.026			0.002			0.008	
Var. x Treat.		0.021			0.037			0.002			0.011	

Sig = Significant; NS = Non-significant

Table 54. Combined effect of jasmonic acid (1.0mM) and aphid infestation (50, 100 and 150 aphids per plant) on total chlorophyll level (mg g^{-1} FM) and carotenoid level (mg g^{-1} FM) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	Total chlorophyll level						Carotenoid level					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	1.720	1.185	1.453	1.827	1.434	1.631	0.570	0.430	0.500	0.540	0.350	0.445
JA + 50 aphids	1.601	1.035	1.318	1.760	1.290	1.525	0.722	0.517	0.620	0.671	0.413	0.542
JA + 100 aphids	1.559	0.998	1.278	1.731	1.251	1.491	0.692	0.493	0.593	0.644	0.392	0.518
JA + 150 aphids	1.513	0.928	1.220	1.695	1.176	1.435	0.664	0.476	0.570	0.622	0.377	0.500
Mean	1.598	1.036		1.753	1.287		0.662	0.479		0.619	0.383	
LSD at 5%												
Varieties		0.012			0.026			0.010			0.018	
Treatment		0.016			0.037			0.014			0.026	
Var. x Treat		0.023			0.053			0.020			0.036	

Table 55. Combined effect of jasmonic acid (1.0mM) and aphid infestation (50, 100 and 150 aphids per plant) on protein content (mg g^{-1} DM) and total phenol content (mg g^{-1} FM) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	Protein content						Total phenol					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	23.36	22.04	22.70	24.27	22.36	23.32	3.82	3.31	3.57	4.53	4.04	4.29
JA + 50 aphids	21.44	19.47	20.45	22.87	20.50	21.68	3.49	2.94	3.22	4.36	3.04	3.70
JA + 100 aphids	19.81	17.98	18.90	21.36	18.97	20.17	3.44	2.78	3.11	4.32	2.97	3.64
JA + 150 aphids	18.09	16.29	17.19	19.42	17.23	18.32	3.40	2.62	3.01	4.26	2.88	3.57
Mean	20.67	18.94		21.98	19.76		3.54	2.91		4.37	3.23	
LSD at 5%												
Varieties		0.135			0.119			0.069			0.132	
Treatment		0.191			0.138			0.097			0.186	
Var. x Treat.		0.270			0.237			0.137			0.263	

Sig = Significant; NS = Non-significant

Table 56. Combined effect of jasmonic acid (1.0mM) and aphid infestation (50, 100 and 150 aphids per plant) on proline content (μ mol g^{-1} FM) and N content (mg g^{-1} DM) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	Proline content						N content					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	13.17	11.28	12.23	15.24	13.69	14.47	5.43	5.20	5.32	6.65	5.46	6.06
JA + 50 aphids	15.27	12.72	13.99	16.44	14.43	15.44	5.32	5.03	5.18	6.60	5.35	5.98
JA + 100 aphids	17.87	13.31	15.59	17.88	14.85	16.37	5.13	4.84	4.99	6.37	5.15	5.76
JA + 150 aphids	21.64	14.93	18.28	20.07	15.79	17.93	4.77	4.71	4.74	5.92	5.01	5.47
Mean	16.99	13.06		17.41	14.69		5.16	4.95		6.38	5.25	
LSD at 5%												
Varieties		0.519			0.454			0.057			0.066	
Treatment		0.734			0.642			0.080			0.094	
Var. x Treat.		1.039			0.906			0.114			0.134	

Table 57. Combined effect of jasmonic acid (1.0 mM) and aphid infestation (50, 100 and 150 aphids per plant) on P and K content (mg g^{-1} DM) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	P content						K content					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	11.29	9.39	10.34	12.96	10.19	11.58	6.13	10.02	8.08	7.47	12.89	10.18
JA + 50 aphids	10.80	8.79	9.79	12.66	9.74	11.20	6.30	10.29	8.29	7.56	13.06	10.31
JA + 100 aphids	9.88	7.67	8.77	11.60	8.54	10.07	6.52	10.55	8.54	7.85	13.41	10.63
JA + 150 aphids	8.88	6.39	7.63	10.46	7.14	8.80	6.60	10.80	8.70	7.94	13.70	10.82
Mean	10.21	8.06		11.92	8.90		6.39	10.41		7.71	13.26	
LSD at 5%												
Varieties		0.106			0.130			0.300			0.314	
Treatment		0.150			0.185			0.424			0.445	
Var. x Treat.		0.212			0.261			NS			NS	

Sig = Significant; NS = Non-significant

Table 58. Combined effect of jasmonic acid (1.0 mM) and aphid infestation (50, 100 and 150 aphids per plant) on number of stomata on abaxial and adaxial leaf surface of *Brassica juncea* cvs. Alaokar and Rohini at 60 and 75 DAS

Treatment	No. of stomata (Abaxial surface)						No. of stomata (Adaxial surface)					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	21.4	18.8	20.1	24.7	22.7	23.7	11.4	10.8	11.1	14.8	12.7	13.8
JA + 50 aphids	21.2	18.3	19.7	24.5	22.2	23.4	11.3	10.5	10.9	14.7	12.4	13.6
JA + 100 aphids	21.2	18.2	19.7	24.5	22.1	23.3	11.3	10.5	10.9	14.7	12.4	13.5
JA + 150 aphids	21.1	18.1	19.6	24.4	22.1	23.3	11.3	10.4	10.8	14.7	12.4	13.5
Mean	21.2	18.4		24.5	22.3		11.3	10.5		14.7	12.5	
LSD at 5%												
Varieties		0.096			0.099			0.074			0.058	
Treatment		0.135			0.139			0.104			0.082	
Var. x Treat.		0.192			0.197			0.147			0.115	

Table 59. Combined effect of jasmonic acid (1.0 mM) and aphid infestation (50, 100 and 150 aphids per plant) on relative stomatal closure index (RSCI) of abaxial and adaxial surface of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	RSCI (Abaxial leaf surface)						RSCI (Adaxial leaf surface)					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
JA + 50 aphids	0.36	0.37	0.37	0.31	0.33	0.32	0.50	0.55	0.53	0.45	0.51	0.48
JA + 100 aphids	0.40	0.42	0.41	0.36	0.38	0.37	0.54	0.61	0.58	0.50	0.57	0.54
JA + 150 aphids	0.51	0.53	0.52	0.45	0.48	0.47	0.62	0.70	0.66	0.57	0.65	0.61
Mean	0.42	0.44		0.37	0.40		0.55	0.62		0.51	0.58	
LSD at 5%												
Varieties		0.005			0.004			0.008			0.006	
Treatment		0.006			0.005			0.008			0.007	
Var. x Treat.		0.008			0.007			0.011			0.010	

Sig = Significant; NS = Non-significant

Table 60. Combined effect of jasmonic acid (1.0 mM) and aphid infestation (50, 100 and 150 aphids per plant) on net phntosynthetic rate (P_N; μ mol CO_2 m^{-2} sec^{-1}) and stomatal conductance (g_s; mol m^{-2} sec^{-1}) of *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	Net photosynthetic rate (P_N)						Stomatal conductance (g_s)					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	10.38	9.32	9.85	12.67	11.19	11.93	40.23	34.89	37.56	46.72	38.42	42.57
JA + 50 aphids	10.72	9.95	10.34	13.05	11.85	12.45	39.92	34.39	37.16	46.54	37.97	42.26
JA + 100 aphids	10.42	9.38	9.90	12.69	11.22	11.96	39.69	34.14	36.92	46.24	37.71	41.98
JA + 150 aphids	9.18	8.48	8.83	10.89	10.03	10.46	39.32	33.90	36.61	45.90	37.50	41.70
Mean	10.18	9.28		12.33	11.07		39.79	34.33		46.35	37.90	
LSD at 5%												
Varieties		0.083			0.133			0.057			0.072	
Treatment		0.117			0.189			0.081			0.102	
Var. x Treat.		0.166			0.267			0.114			0.144	

Table 61. Combined effect of jasmonic acid (1.0 mM) and aphid infestation (50, 100 and 150 aphids per plant) on pod length (cm) and oil content (%) of *Brassica juncea* cvs. Alankar and Rohini at harvest (120 DAS)

	Pods length			Oil content		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	5.02	3.91	4.47	36.41	32.85	34.63
JA + 50 aphids	4.89	3.77	4.33	36.10	32.41	34.25
JA + 100 aphids	4.55	3.47	4.01	35.73	32.04	33.88
JA + 150 aphids	4.28	3.16	3.72	35.19	31.53	33.36
Mean	4.68	3.58		35.85	32.21	
LSD at 5%						
Varieties		0.011			0.575	
Treatment		0.016			0.813	
Var. x Treat.		0.022			0.701	

Sig = Significant; NS = Non-significant

Table 62. Combined effect of jasmonic acid (1.0 mM) and aphid infestation (50, 100 and 150 aphids per plant) on yield characteristics of *Brassica juncea* cvs. Alankar and Rohini at harvest

	Pods plant^{-1}			Seeds pod^{-1}			1000 seeds weight (g)			Seed yield (g)		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	109.64	87.43	98.54	11.96	10.79	11.38	5.51	4.26	4.89	7.23	4.02	5.62
JA + 50 aphids	102.59	80.21	91.40	11.30	10.05	10.67	5.21	3.79	4.50	6.04	3.05	4.55
JA + 100 aphids	89.18	69.59	79.38	10.51	8.75	9.63	4.91	3.32	4.11	4.60	2.02	3.31
JA + 150 aphids	74.48	58.24	66.36	9.81	7.65	8.73	4.52	3.08	3.80	3.30	1.37	2.34
Mean	93.97	73.87		10.89	9.31		5.04	3.61		5.29	2.62	
LSD at 5%												
Varieties		1.638			0.238			0.076			0.156	
Treatment		2.316			0.351			0.107			0.221	
Var. x Treat.		3.276			0.496			0.152			0.312	

Table 63. Combined effect of jasmonate (1.0 mM) and aphid infestation (50, 100 and 150 aphids per plant) on the aphid count and number of beetles attracted on *Brassica juncea* cvs. Alankar and Rohini at 60 and 75 DAS

Treatment	Aphid count						Number of beetles attracted					
	60 DAS			75 DAS			60 DAS			75 DAS		
	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean	Alankar	Rohini	Mean
Control	0	0	0	0	0	0	0	0	0	0	0	0
JA + 50 aphids	47	51	49	85	102	94	11	9	10	4	3	4
JA + 100 aphids	96	104	100	142	157	150	15	12	14	7	6	7
JA + 150 aphids	161	171	166	210	221	216	13	10	12	5	4	5
Mean	76	82		109	120		10	8		4	3	
LSD at 5%												
Varieties		1.689			2.383			0.368			0.155	
Treatment		2.388			3.370			0.520			0.219	
Var. x Treat.		3.378			4.766			0.735			0.310	

Sig = Significant; NS = Non-significant